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RECOGNITION OF THE ALLERGIC STATE BY TISSUE EXAMINATION

The Respiratory Tract and the Nasal Sinuses*

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The scope of the subject presented in this work is limited to the types of allergic disease which Coca labelled "atopic" and which corresponds to certain forms of human hypersensitiveness with an hereditary basis such as asthma and hay fever. There are several shock organs (as Doerr designated those organs which show the allergic lesions) in human hypersensitiveness consisting of the skin, the conjunctiva, the nose, the lung, the gastro-intestinal tract, and possibly the meninges, the urinary bladder and the retina. Of these organs the basic tissues of the more commonly encountered diseases may be placed into three groups: (1) the mucosa of the respiratory tract and the accessory nasal sinuses, (2) the skin and (3) the mucosa of the gastro-intestinal tract. Probably and largely because of the difference in the histological structure of these tissues, there is a modification in their allergic pathological picture. The work here presented will consider the histopathology of the respiratory mucosa and that of the accessory nasal sinuses as exemplified by the clinical conditions of allergic asthma, hay fever and sinusitis.

The literature contains reports of forty-seven necropsies performed on people dying of a presumptive allergic asthma. The earlier reports were summarized by Huber and Koessler[3] and later contributions were made by Kountz and Alexander,[4] Steinberg and Figley,[7] and Macdonald[5] with references to other reported cases. Critical analyses of these forty-seven necropsy reports

* Read before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9 to 12, 1933.

(Huber and Koessler, Steinberg and Figley, and Rackemann[6]) indicate that only some sixteen of them were actually instances of allergic asthma and a still smaller number were free of complicating conditions which might have altered and confused an unfamiliar pathological picture. Those who had the opportunity to study at first hand the morbid anatomy of cases of true allergic asthma were uniformly impressed with the peculiar pathologic changes in the lungs. Either by the implied written word or by personal communication to the author, some of these observers expressed their belief that there is a distinct histopathological picture associated with allergic asthma. If, however, a survey is made of all the reported cases without regard to their etiological basis, a contrary view may be formulated. Walzer[8] analyzed the pathological findings of thirty-three cases and concluded that there is no uniformity in the findings nor is there a clue to the pathology of bronchial asthma and that the changes found are due to secondary bronchiectasis and emphysema. This view is not in accord with that expressed by most of the other observers.

Hansel[1] reported that the pathological changes in the nasal mucosa were similar to those in the bronchi of asthmatic individuals. Kountz and Alexander removed a section of the nasal mucous membrane from one of their cases of allergic asthma and found that the histopathological picture there and in the bronchi were alike. These observations explain the frequent clinical manifestations of sneezing and coryza preceding an asthmatic attack. Matson in discussing the presentation of Steinberg and Figley stated that the tissue of the accessory nasal sinuses in asthmatics contained eosinophiles and edema with an hyperplasia of the lining epithelium including goblet cells. Tissue removed from all the accessory sinuses of the nose in one of my cases of allergic asthma presented pathological changes in the nose similar to those observed by Hansel and Kountz and Alexander. Hansel[2] studied the histopathology of the nasal and sinus mucosa in hay fever, vasomotor rhinitis and bronchial asthma. The changes he observed in the three conditions were identical. It is therefore apparent that in the individuals with allergic asthma,

the entire respiratory tract including the sinuses participate in the altered histological structure and that these changes throughout the system are similar in character. It may also be inferred that the allergic (atopic) state, in whatever manifestation it occurs in affecting the respiratory and sinus mucosa (asthma, hay fever, sinusitis, rhinitis) is characterized by a single and a constant histopathological picture. The purpose of this work is to present this histopathology and further evidence to substantiate these contentions. The work is based on a study of three necropsies on people dying of allergic asthma and an analysis of true allergic asthma cases in the literature. The study also includes the examination of accessory sinus tissue from thirty cases of sinusitis including nasal tissue in those with hay fever.

PATHOLOGY OF LUNG IN ALLERGIC ASTHMA

The three cases of allergic asthma which form the basis of the pathological description to follow were definitely established instances of allergy as determined by immunological and hereditary criteria. Two other cases were left out of consideration because they lacked either the immunological or the hereditary evidences and also because of complicating infectious manifestations. The lungs only will be described since the other organs did not reveal morbid changes which could be attributed to allergic disease.

Gross appearance

The lungs were voluminous but were decreased in weight to a little more than half of the normal. The surface lobules were enlarged. In the case of long standing disease, there were patches of bullous emphysema. The cut surface was dry, very spongy with large visible alveoli. An occasional area was of a dull red color, the alveoli were obliterated and the tissue was rubbery to touch. These were areas of atelectasis. The walls of all the bronchi were perceptibly thickened and were gray white in color. The lumina of the bronchi were filled with gray, frequently concentrically arranged plugs. The consistency of these plugs varied. They were soft, mucoid and fairly easily removable

but more frequently they were stony hard and could not be dislodged. Bronchi of every calibre were involved.

Histological appearance

The lining cells of the bronchi which were normally ciliated, columnar in type with interspersed goblet cells, had undergone several changes. In the bronchi with hard plugs, the columnar cells were reduced to very short cuboidal with dense nuclei which occupied the entire cell. The goblet cells were few in number or entirely absent. The bronchi with soft plugs or with a lumen incompletely filled had several layers of columnar epithelium with numerous goblet cells. The nuclei of the columnar cells were located at the margins of attachment, they were vesicular and showed prominent nucleoli. The goblet cells were distended with circular or ovoid areas either clear or containing mucinous material.

The basement membrane was considerably thickened and structureless in character. It gave a hyaline reaction to Unna's stain. The average width of the basement membrane was 0.025 mm. irrespective of the calibre of the bronchus. The basement membrane of the smaller bronchi was of the same width or wider than that of the larger bronchi. The mucosa contained a variable degree of edema and cellular infiltration. Eosinophiles composed from 15 to 85 per cent of all cells, the remaining were lymphocytes. There was a varying proportion of the mononuclear and the polymorphonuclear eosinophiles with a tendency to a greater number of the mononuclear type. The muscle layer was also infiltrated by similar cells. There was a separation of the muscle bundles by the edematous fluid. The muscle tissue was distinctly increased in amount. Measurements demonstrated their increase to be from five to ten times that of normal. There were no apparent changes in the muscle fibers except for a possible increase in width and more than the usual vesicular nuclei. The musculature was smaller in amount in cases in which the disease was of a brief duration. The elastic fibers appeared broken.

The mucous glands for the greater part were markedly increased in size and were filled with a large amount of mucus. The

diameter of an average gland was 0.18 mm. as contrasted with 0.058 mm. for a normal. The cells composing the mucous glands were indistinguishable with only an occasional outline to indicate the presence of a cell wall. The nuclei were dense, flattened and were crowded to the base of the cell. An occasional basement membrane was thickened and hyalinized. There was an apparent hypersecretory activity of the glands but the changes in the cytoplasmic structure were probably reversible since the nuclei though compressed did not show disintegrative changes. The tissue surrounding the glands was edematous and contained a cellular exudate similar to that in the mucosa. The cartilage

CHART 1

A Summary of the Essential Changes Characterizing the Histopathology of the Lungs in Allergic Asthma

1. Emphysema.
2. Edema of the bronchial wall.
3. Hypertrophy of the bronchial muscle.
4. Eosinophilic infiltration of the bronchial wall.
5. Hypertrophy and hypersecretory activity of the mucous glands.
6. Increased amount of mucus in bronchial and gland lumina.
7. Hypertrophy and hyalinization of the basement membrane.
8. Hyperplasia and hypersecretory activity of the goblet cells of the bronchi.
9. Degenerative changes of the cartilage cells of the bronchi.

plates showed degenerative changes with decalcification in some places and an apparent increase in the calcium in others. The lumina of the bronchi contained strands and structureless masses of mucus with enmeshed desquamated epithelial cells, lymphocytes and eosinophiles. In some of the bronchi, especially those with cuboidal cells, the lumen contents were adherent to the lining cells. There were frequent herniations into the wall of the bronchi. The lumina were actually increased in diameter as demonstrated by comparative measurements with normal bronchi. This increase was apparently due to an excessive amount of mucus secreted into the lumen. Although there was an actual increase in the diameter of the bronchial lumen, there was no functional advantage because of the plugging by mucus.

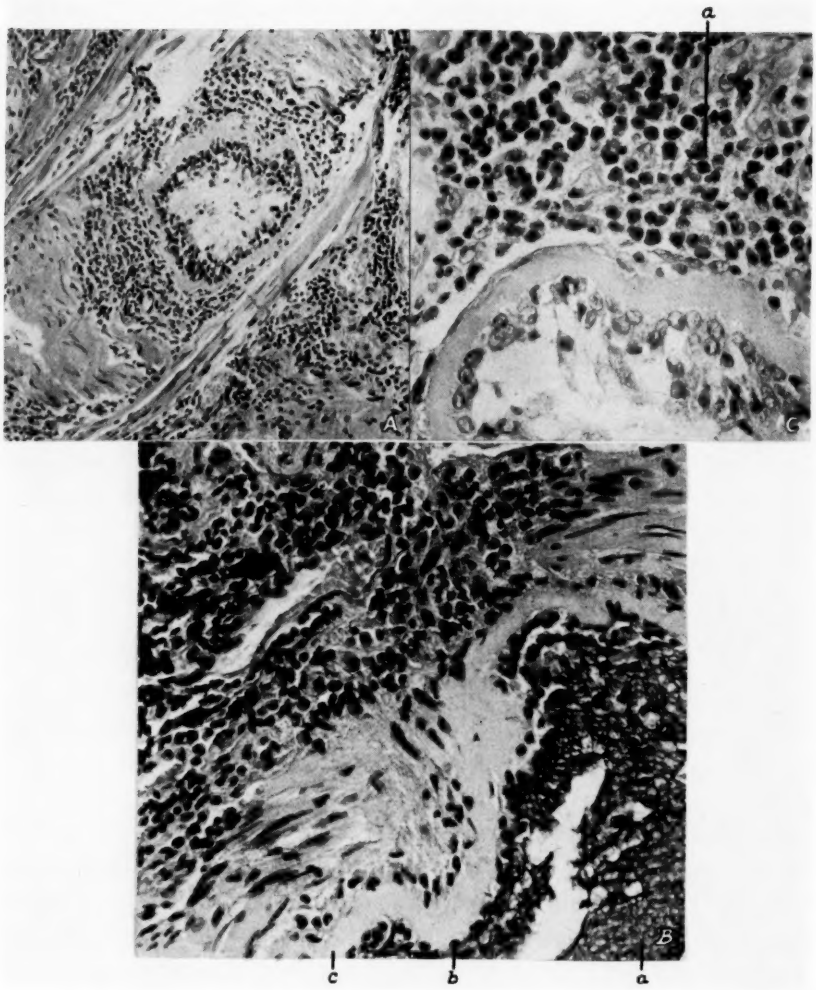
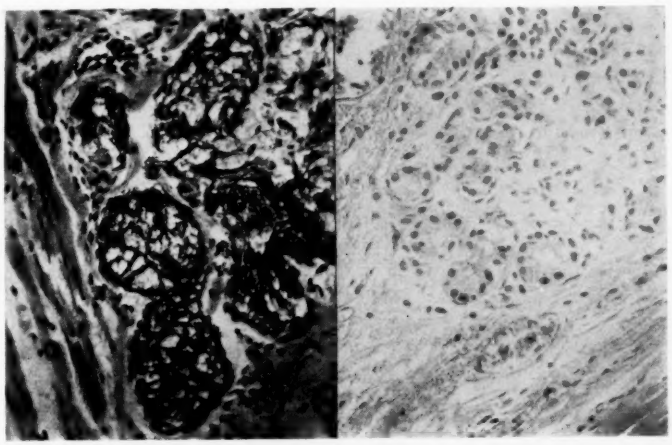


FIG. 1. A. Section of lung in allergic asthma. A bronchus filled with a moderate amount of mucus. Hyperplasia of the lining cells. Thickened and hyalinized basement membrane. Cellular infiltration, the cells are predominantly eosinophilic. Hypertrophy of the muscular structure.

B. A higher power photograph of a part of a bronchus from a case of allergic asthma. a. Mucous secretion in the lumen. b. Cuboidal lining cells. c. Thickened and hyalinized basement membrane.

C. A high power photograph of a part of a bronchus from a case of allergic asthma. a. An eosinophile representing 80% of the total number of infiltrating cells.

The alveoli were distended and many of the septa were broken allowing many alveoli to intercommunicate. The septa were considerably narrower than normal. The capillaries and the blood vessels were dilated and contained large amounts of blood. This emphysema was apparently of long standing. The essential changes are summarized in chart 1.



A B

FIG. 2. A. Mucous glands from the wall of a bronchus of a lung in a case of allergic asthma. The glands are increased in size. The lining cells are not apparent. In place of the cells there is a considerable amount of mucus which also fills the lumen. There is a cellular exudate surrounding the glands. The cells are predominantly eosinophilic.

B. Mucous glands from the wall of a bronchus in a case of chronic bronchitis. The glands are normal in size. The lining cells are distinct. The nuclei are preserved. There is no mucus in the lumen.

PATHOLOGY OF THE MUCOUS MEMBRANES OF THE NOSE AND THE ACCESSORY SINUSES IN HAY FEVER AND ALLERGIC SINUSITIS

The clinical histories and the pathological findings were correlated in thirty cases in which the sinus and in many the nasal tissue were removed. In more than half of the cases, the sinus operation was performed in several stages allowing a study of the tissue during various stages of activity of the disease. Because

the histological structure and the pathological changes are alike, the sinus and nasal tissue will be considered together.

Gross appearance

The tissue was boggy and occasionally polypoid. The color was a muddy gray pink. There was frequently a thick, gray,

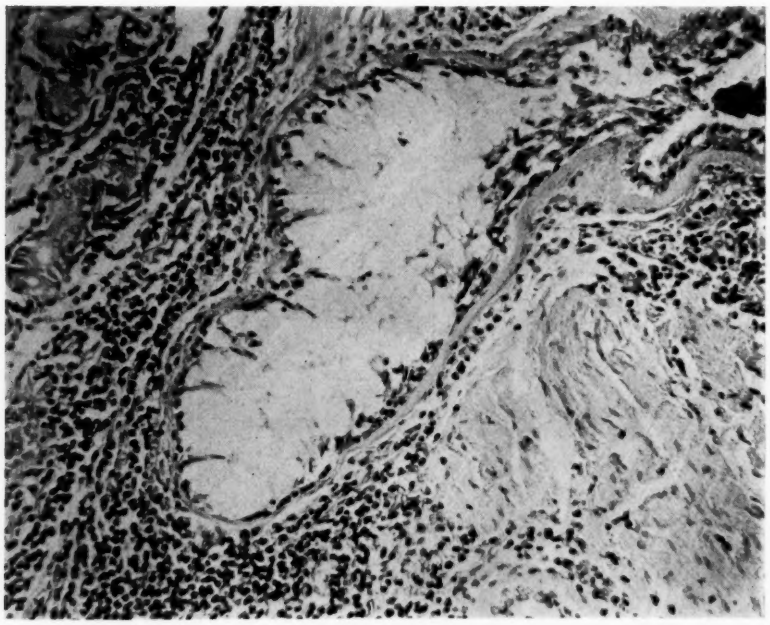


FIG. 3. A BRONCHUS FROM A LUNG FROM A CASE OF ALLERGIC ASTHMA. HERNIATION OF A BRONCHUS INTO THE WALL

The lumen is filled with mucus. The lining cells are cuboidal in type. There are outlines of numerous goblet cells. The basement membrane is thickened and hyalinized. The cellular infiltration consists of eosinophiles and lymphocytes.

mucoid substance on the surface and occasionally streaky gray mucus in strands or small masses. The cut surface was gray, glarey, glistening and very moist. There were small pin points of darker gray areas which correspond to a group of hypertrophied tubules.

Histological appearance

The lining cells which are normally ciliated columnar were frequently reduced to low cuboidal. The nuclei were dense and were located at the attached margin of the cell. Associated with this change of the epithelium, there was a complete disappearance of the goblet cells. In other instances and frequently in the same field there was a marked increase in size and in number of the goblet cells with preservation and hyperplasia of the ciliated columnar epithelium which was frequently present in two or three layers. The goblet cells were distended with mucus. The nuclei were elongated and compressed to one side of the cell wall. The basement membrane was thickened and hyalinized. The average variation in the width was 0.1 to 0.2 mm. Parts of the membrane in the same tissue remained normal and the width varied in the same microscopic field. In the clinically early cases of recent origin, there was thickening but preservation to some extent of the fibrillar structure. In the older cases, the fibrils had disappeared and the membrane presented a homogeneous appearance.

The mucous membrane was edematous. The edema varied proportionally with the acuteness of the condition. The fluid had a bluish tinge in a hematoxylin-eosin preparation. The fibroelastic tissue was compressed and in places was granular. There was a variable cellular infiltration. The greater number of cells were eosinophiles with a variable proportion of the polynuclear and the mononuclear types. The eosinophiles constituted from 15 to 90 per cent of all cells, the number depending upon the acuteness and the severity of the clinical state. Lymphocytes, endothelioid cells and an occasional plasma cell composed the remaining cellular constituents. Fibroblastic proliferation was present to a very slight extent and in a very few instances.

The mucous glands were increased in size and were present in apparent larger numbers. The average individual hyperplastic gland measured 0.15 mm. as contrasted to the dimensions of a normal of 0.06 mm. The glandular lumen was dilated and filled with mucus. The lining cells were for the greater part not ap-

parent and only a thin line showed the presence of the cell wall. Some of the glands contained a fairly well defined cell wall and the cytoplasm had globules of mucus. The nuclei were located

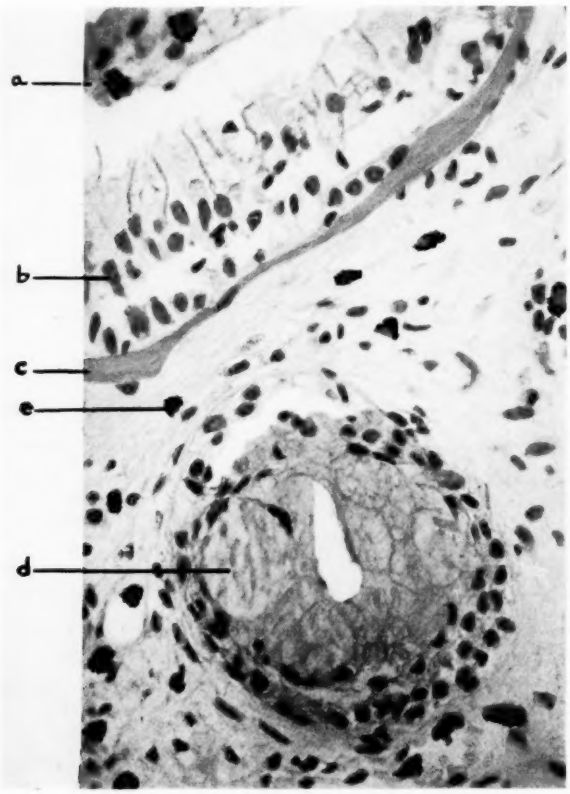


FIG. 4. FRONTAL SINUS TISSUE FROM A CASE OF ALLERGIC SINUSITIS WITH A HISTORY OF ALLERGIC ASTHMA

a—Lumen of sinus containing mucus and eosinophiles. b—Several layers of lining cells and goblet cells. c—Thickened and hyalinized basement membrane. d—A mucous gland which is increased in size, but the lumen contains a large amount of mucus and flattened lining cells. e—Mucosa with edema and eosinophilic infiltration.

at the base of the cell, they were compressed and were stained deeply. The cartilage cells took a deep hematoxylin stain in

some places and in others there was an evident diminution of calcium. The cytoplasm and the nucleus showed degenerative changes.

An analysis of the clinical state of the patient and the correlation to the histopathology showed evidence that the histological changes vary with the clinical state of the patient. These variations are shown in chart 3.

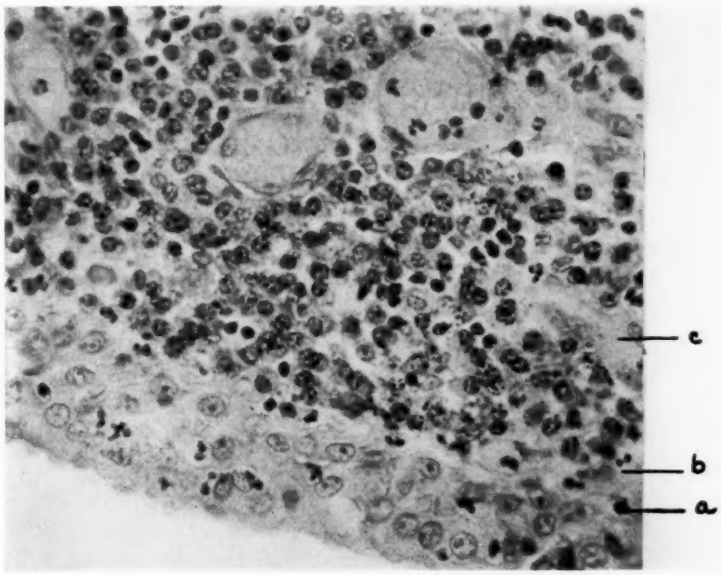


FIG. 5. TISSUE FROM THE FRONTAL SINUS FROM A CASE OF CHRONIC INFECTIOUS SINUSITIS

a—Several layers of lining epithelium which tend to assume a squamous type. b—An indistinct basement membrane. c—A mucosa diffusely infiltrated with plasma cells and a sprinkling of polymorphonuclears. Contrast this histopathological picture with that in Figure 4 representing an allergic state.

SUMMARY

There is a distinct histopathological picture of the mucosa of the entire respiratory tract and of the accessory nasal sinuses associated with the allergic (atopic) state. The morbid changes are of a similar nature in the atopic conditions of asthma, hay

CHART 2

A Summary of the Essential Changes Characterizing the Histopathology in Allergic Sinusitis and Hay Fever

1. Edema.
2. Hyperplasia and hypersecretory activity of the goblet cells.
3. Thickening and hyalinization of the basement membrane.
4. Eosinophilic infiltration.
5. Hypertrophy and hypersecretory activity of the mucous glands.
6. Presence of mucus in lumen of sinus and glands.

CHART 3

Histopathology of the Various Stages of Allergic Sinusitis

STAGE	MUCOUS GLANDS	EDEMA	GOBLET CELLS	BASEMENT MEMBRANE	AMOUNT OF MUCUS	EOSINOPHILIA
Acute Stage	Hyperplasia Hypertrophy Hypersecretory activity	Moderate to marked	Hyperplasia Hypersecretory activity	Slightly thickened, granular or homogeneous	Moderate to marked	75–90 per cent
Chronic Stage	Hyperplasia Hypertrophy Hypersecretory activity and dilatation of glandular lumina	Moderate to marked	Hyperplasia Hypersecretory activity	Greatly thickened and homogeneous	Moderate to marked	35–90 per cent
Remission	Hyperplasia Little or no hypertrophy No secretory activity	Very slight	Not apparent	Moderately to greatly thickened and homogeneous	Little or none	15 per cent

CHART 4

Summary of the Essential Histopathological Characteristics of Allergic (Atopic) Mucous Membranes of the Entire Respiratory Tract and Accessory Nasal Sinuses

1. Hypertrophy and marked secretory activity of the mucous glands.
2. Presence of large amount of mucus in lumina.
3. Eosinophilia—from 15 to 90 per cent of all cells.
4. Edema of tissue.
5. Thickening and hyalinization of the basement membrane.
6. Hyperplasia of goblet cells with hypersecretory activity.

fever and rhinitis (chart 4). In asthma, in addition to the lungs, the rest of the respiratory tract including the nose and almost invariably the accessory sinuses show these morbid changes. This constant pathological picture of the respiratory, nasal and sinus mucosa permits recognition of the allergic (atopic) state involving these organs.

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THE SPECIFICITY OF THE TEST FOR ALCOHOL IN BODY FLUIDS*

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The subject of alcohol and its effects on the human body has recently assumed unusual interest, and the public has been so overwhelmed with political and business propaganda that the results of scientific investigation have been thrust into the background. The question has assumed such emotional characteristics that it provokes reactions similar to those accompanying discussions of politics and religion.

Even the diagnosis of drunkenness is no longer limited to the medical profession, a supreme court having ruled that any person of ordinary intelligence is competent to testify as to the intoxication or sobriety of a person. This is an indictment of the medical profession, in recognition of the fact that its members have failed to diagnose drunkenness to the satisfaction of courts and juries. Even though the accused may have been visibly intoxicated, a clever lawyer can point out that there is no symptom of alcoholic intoxication which may not be simulated by some other pathological condition, and acquittal usually follows.

Many observers, among whom are Nicloux[1] and Nowicka,[5] Widmark,[7] Southgate[3] and Carter,[6] Bogen,[1] McNally and Embree[4] have shown that a chemical test for alcohol furnishes the only constant finding in all cases of alcoholic intoxication, and also allows the examiner to state with assurance that at least a certain amount of alcohol was taken into the body. We[3] have also confirmed the fact that the intensity of intoxication fairly closely parallels the per cent of alcohol in the blood or urine, regardless of tolerance, age, weight, or food consumption. This was shown by

* Read before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9 to 12, 1933.

controlled experiments on human beings, and practical confirmation was furnished by my co-worker Dr. Benjamin Halporn, who was able to estimate the amount of alcohol in the urine of persons accused of driving while drunk with remarkable accuracy from the symptoms alone, his estimate being later checked by chemical analysis.

Although the test has been used by us chiefly as a means of confirming evident drunkenness, it also proves of great value in collecting information about individuals who show no symptoms of intoxication, having been suddenly sobered by an accident or contact with the police. Also cases in which the injuries of persons mask the effects of alcohol, and cases of fatal accidents will yield valuable information on chemical analysis for alcohol.

We have had several cases in which the chemical test aided in the differential diagnosis of coma, one of which will be summarized.

A middle aged man was sent to the hospital in a state of coma. According to the history given by relatives he had been drinking and had fallen striking his head. The x-ray revealed a fracture through the parietal bone and right mastoid. The condition of the patient was alarming and the surgeon wished to know whether or not to operate. A catheterized specimen of urine obtained two hours after the accident revealed 0.42 per cent alcohol, and spinal fluid examined one hour later revealed a pressure of 300 mm. water, normal rise on jugular compression; three lymphocytes and 20 erythrocytes; globulin ++; and alcohol 0.38 per cent. The conclusion that alcohol could have been responsible for most of the alarming symptoms was later on justified, the patient making a rapid recovery.

So meager is the information regarding alcohol flavored accidents, that the state of Pennsylvania reports drunkenness as associated with fatal auto accidents as but 1 per cent for 1931. These figures have suggested that the rôle of alcohol has not been properly evaluated, and we are now engaged in a survey to determine the relationship of alcohol to auto accidents, having succeeded in collecting data on fifty accidents (table 1). While the number of cases is, of course, too small to permit drawing of conclusions, it at least suggests the importance of a nation-wide survey.

Obviously such work would be useless unless we had a simple,

TABLE 1

PRELIMINARY INVESTIGATION OF FIFTY CONSECUTIVE AUTOMOBILE ACCIDENTS

	NUMBER	INJURED	KILLED
"Alcohol accidents"	32	71	4
No alcohol involved	18	23	0

TABLE 2

STRONG PERMANENT STANDARDS

PER CENT ALCOHOL BY WEIGHT	ALCOHOL BY WEIGHT	WATER
		cc.
0.0		1.00
0.05	0.50 cc. of 0.10 per cent	0.50
0.10	1.00 cc. of 0.10 per cent	0.0
0.12	0.60 cc. of 0.20 per cent	0.40
0.14	0.70 cc. of 0.20 per cent	0.30
0.16	0.80 cc. of 0.20 per cent	0.20
0.18	0.90 cc. of 0.20 per cent	0.10
0.20	1.00 cc. of 0.20 per cent	
0.22	0.73 cc. of 0.30 per cent	0.27

To each tube add 3 cc. N/15 $K_2Cr_2O_7$ (0.33 per cent in 50 per cent H_2SO_4). Place tubes in boiling water four minutes and seal.

TABLE 3

WEAK PERMANENT STANDARDS

PER CENT ALCOHOL BY WEIGHT	ALCOHOL BY WEIGHT	WATER
		cc.
0.0		2.0
0.005	1.0 cc. of 0.01 per cent	1.0
0.010	2.0 cc. of 0.01 per cent	0.0
0.013	0.52 cc. of 0.05 per cent	1.48
0.016	0.64 cc. of 0.05 per cent	1.36
0.019	0.76 cc. of 0.05 per cent	1.24
0.022	0.88 cc. of 0.05 per cent	1.12
0.025	1.00 cc. of 0.05 per cent	1.00
0.028	1.12 cc. of 0.05 per cent	0.88
0.031	1.24 cc. of 0.05 per cent	0.76
0.034	1.36 cc. of 0.05 per cent	0.64
0.037	1.48 cc. of 0.05 per cent	0.52
0.040	1.60 cc. of 0.05 per cent	0.40

Add 1 cc. $K_2Cr_2O_7$ reagent to each tube. Place tubes in boiling water bath twelve minutes.

specific test, and could be assured that the alcohol in specimens would not change appreciably for several days. Many methods for determining alcohol have been used, but one of the simplest is the reduction of potassium dichromate with a change of color from orange to blue, the reading being obtained by comparing the color with standards prepared by adding known amounts of alcohol to the reagent. The technic previously published has been improved.

TECHNIC OF TEST

Distill mixture of 10 cc. of urine with about 10 cc. of half saturated picric acid containing about 10 per cent tartaric acid, collecting the first 10 cc. of the distillate and mix. In separate tubes similar to those used for the standards, place 1 cc. in one, and smaller measured amounts in the others, making the volume up to 1 cc. in each case. Add 3 cc. of the $K_2Cr_2O_7$ reagent to each tube, and place in boiling water bath four minutes. Compare colors with those of the standard scale. Divide the reading by the fraction of a cubic centimeter of distillate used, which gives the percentage of alcohol by weight. The use of several tubes permits close checking of the results and gives greater opportunity for having readings on the scale.

If results are too low to be read, use the weak standards, using 2 cc. of the distillate and known smaller amounts, bringing the volume to 2 cc. in each case, add 1 cc. of the reagent and place tubes in boiling water twelve minutes.

If blood is being tested take 2 cc. of whole blood, plasma, or serum (all give the same results), add about 15 cc. of the picric-tartaric reagent, and collect the first 10 cc. of the distillate. This is tested on the weak standard scale and the result mutiplied by 5.

ACCURACY OF TEST

The color changes produced are so definite that two technicians can consistently check results within 0.01 per cent, when the strong standards are used, and 0.002 per cent when the weak standards are used.

Using a 500 cc. Pyrex Florence flask, heating with a flame through an asbestos pad, and using a vertical condenser, not of the spiral type, no measurable loss of alcohol could be found after distillation.

SPECIFICITY OF TEST

Mention is frequently made of traces of a reducing substance found normally in body fluids, which might be confused with

alcohol. This substance has recently been proved to be ethyl alcohol by Gettler[2] and our experiments on non-drinkers have consistently shown the amount to be below 0.005 per cent.

Authors have repeatedly pointed out that the reduction of potassium dichromate is not necessarily specific for alcohol, and have named ether, salicylic acid, chloral, chloroform, acetone and lactic acid as possible sources of error.

Our previous paper has given results of experiments which indicated that chloroform, ether, chloral hydrate, salicylates, and acetone could not be mistaken for ethyl alcohol, since they could not be present in sufficient amounts to appreciably reduce $K_2Cr_2O_7$ and, furthermore, gave high readings with the refractometer thus giving evidence of their presence.

The fact that ether does not interfere with the test is further demonstrated by testing the blood from a patient who had been having an ether anaesthetic for one hour. Here the refractometer reading corresponded to 0.03 per cent of alcohol, but the chemical test read 0.002 per cent.

Lactic acid readily reduces $K_2Cr_2O_7$ and does not have as high a refractive index compared to its reducing properties as the other substances studied. For example 1 per cent lactic acid by weight corresponds to 0.60 per cent alcohol by the reduction test and to 1.97 per cent alcohol when tested with the refractometer. However this substance does not appear in the distillate, and need not be considered as a source of error.

Salicylates failed to appear in the distillate and can also be disregarded.

Our experiments have clearly shown that none of the substances said to interfere have any practical bearing on the specificity of the test. Also the use of the refractometer may be omitted, although it is a convenient method of checking the results, and is a particularly efficient weapon to confound the attorney for the defense who has been delving into the literature where he has found no mention of this instrument.

PRESERVATION OF SPECIMENS

We have already shown that specimens of urine, even from diabetics, show no appreciable change in alcohol content for at

least twenty-four hours when left at 37.5°C. Also specimens preserved with benzoic acid retain their alcohol for months, and no fermentation is produced even in the presence of dextrose and yeast.

Continuing these experiments with blood, it was found that blood containing potassium oxalate, sodium citrate, and benzoic acid remained unchanged as far as alcoholic per cent was concerned for at least five days. Sodium fluoride proved to be the best preservative, the alcohol decreasing only from 0.30 to 0.28 per cent in a month, and showing no change for ten days. All specimens were kept at room temperature 20° to 30°C. (see table 4).

TABLE 4

KEEPING PROPERTIES OF BLOODS TO WHICH HAVE BEEN ADDED PRESERVATIVES AND ANTI-COAGULANTS

(Readings represent alcohol in per cent)

DATE	SODIUM FLUORIDE	POTASSIUM OXALATE	SODIUM CITRATE	BENZOIC ACID
April 25	0.30	0.30	0.40	0.37
April 27	0.30	0.30	0.40	0.37
April 29	0.30	0.30		
May 1	0.30	0.30	0.40	0.35
May 5	0.30	0.10	0.40	0.35
May 11	0.28		0.28	0.30
May 22	0.27		0.008	0.005
May 27	0.28			

SUMMARY AND CONCLUSIONS

A simple and specific test for alcohol in body fluids is described and its specificity has been demonstrated.

Specimens of blood and urine may be preserved for at least a month. For blood use sodium fluoride, and urine, benzoic acid.

The importance of the test lies in its ability to confirm a diagnosis of drunkenness for medicolegal purposes, as well as to give valuable information in differential diagnoses.

A preliminary survey of persons injured or killed in auto accidents, suggests that alcohol may be a greater factor in such accidents than statistics indicate, and shows the importance of a

nation-wide survey of the relationship of alcohol to automobile accidents.

The chemical test for alcohol in body fluids will be an important factor in arriving at conclusions concerning the intoxicating ability of certain beverages.

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THE PATHOGENESIS OF NEUTROPENIA

A Theoretical Consideration*

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A decrease or absence of neutrophilic leukocytes in the blood stream has been a subject of growing importance during the past thirty-two years. However, until 1922, neutropenia had been recognized only as a part of certain well known clinical syndromes. Then it was recognized by Schultz as a rapidly fatal symptom complex which included marked prostration, peripheral neutropenia, and a severe grade of progressive oral sepsis. In the past eleven years, much additional information has been gained, but the etiology still remains a baffling problem. The rapidly increasing literature has brought out two important observations which have tended to eliminate the most widely accepted hypothesis of pathogenesis, that is, that the neutropenia is secondary to a septic or toxic process which causes an aplasia of the myeloid tissue of the bone marrow. The first observation shows that the neutropenia may definitely precede the appearance of infection.[22, 25, 9, 4] The second observation demonstrates that myeloid aplasia does not always accompany neutropenia. It is now suggested that the condition of the myeloid tissue may not be the primary pathologic mechanism of this disease.

In approaching the consideration of an hypothesis to attempt to explain the mechanism of neutropenia, it will be necessary to consider briefly the pathology and physiology of the myeloid tissue in reference to the neutropenic state, and to think of the myeloid tissue as constituting an organ with all the potentialities for hypertrophy, atrophy, and functional insufficiency that appertain to any other organ.

* Read before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9 to 12, 1933.

The use of a simple diagram (fig. 1) will assist in making the discussion more intelligible. The myeloid tissue of the bone marrow is represented by a white cone shaped area. The line A, drawn across the cone, indicates the normal level at which the myeloid tissue functions, which will be represented as ++. The area to the right will represent the blood stream in which is a neutropenia.

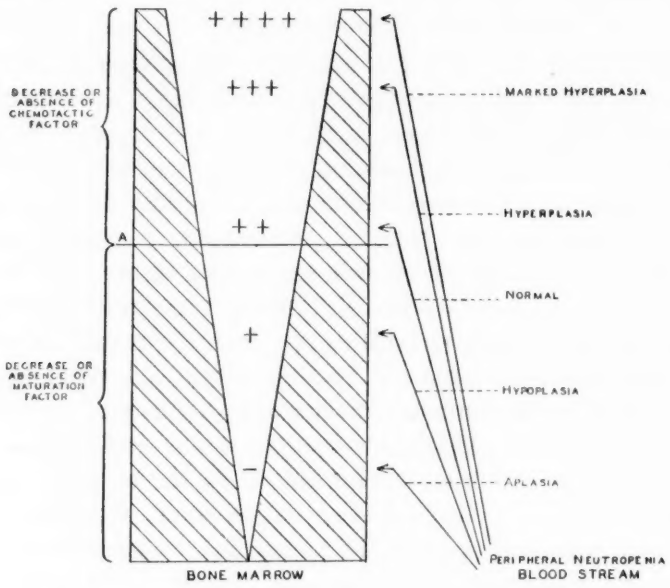

FIG. 1

When one finds the neutrophilic cells greatly reduced in the blood stream, that is, when there is a peripheral neutropenia, the first question which comes to mind is; what is the pathology in the bone marrow where these cells are formed?

When these cases were first being reported and autopsies obtained, the assumption that myeloid aplasia was the pathology underlying this disease seemed to be substantiated, as that was the condition found.[22, 20, 14, 1, 23] This "aplasia concept" had developed partly on this basis of studies of bone marrow taken at autopsy, and partly on the basis of analogy to established findings in certain other conditions, such as in benzol poisoning, et cetera.

Necropsies on all of those patients having peripheral neutropenia disclosed myeloid aplasia, and until 1929, this question seemed to be settled. I will represent this myeloid aplasia by a minus sign at the bottom of the cone.

In 1929, Buck[6] published the report of a patient who had peripheral neutropenia while biopsy of the sternal marrow showed normal myeloid tissue. The necropsy diagnosis on the bone-marrow was "acute hematopoiesis." This same case was included with a series reported by Dameshek and Ingall.[8] Baldridge and Needles,[3] reported a case which had lasted over a period of about four years. They finally resorted to splenectomy as a possible cure. There was not the usual rise of leukocytes after the operation as in other cases, but there was the usual platelet response. The patient died thirty-five days after the operation. The bone-marrow at autopsy disclosed an overgrowth of myelocytes and myeloblasts almost as marked as in myelogenous leukemia. This added to the records another case with peripheral neutropenia and a marked myeloid hyperplasia. However, this result was considered unsatisfactory because a biopsy of the bone-marrow was not obtained before splenectomy, and it was felt that the splenectomy may have played some part in the myeloid hyperplasia. The true status of these findings in fatal cases was substantiated later, however, when Fitz-Hugh and Krumbhaar[15] reported three cases of neutropenia, one of which showed a marked myeloid hyperplasia at necropsy. The other cases were said not to be aplastic. The fact was then established that marked myeloid hyperplasia could underlie peripheral neutropenia. This marked myeloid hyperplasia is represented in the diagram by the $++++$ signs.

Rosenthal[24] reported data on a group of patients having benign neutropenia (patients who recovered) on two of whom biopsies of sternal marrow were made. In these patients he found normal or hyperplastic myeloid tissue. This finding added to our group cases of recovery from peripheral neutropenia with normal or moderately hyperplastic myeloid tissue. This moderately hyperplastic myeloid tissue is represented in the diagram by $+++$.

There are other cases reported in which the patients seem to

have normally a leukopenia and neutropenia,[24] and some patients who have what is called a fifty-fifty differential count; for example 45 per cent neutrophiles and 45 per cent lymphocytes. The myeloid tissue, in these patients, is evidently functioning at a lower level, although I do not have biopsies on bone-marrow to substantiate this. Roberts and Kracke[22] stated "These are the patients who are most likely to develop a severe neutropenia." I represent this type of myeloid tissue in the diagram by +.

The fact is now established that with peripheral neutropenia there can be almost any type of myeloid tissue from aplastic to markedly hyperplastic. To summarize: deaths have occurred with peripheral neutropenia and myeloid tissue of aplastic to markedly hyperplastic nature; recoveries have occurred with peripheral neutropenia and normal or hyperplastic myeloid tissue. The only condition not reported is recovery with a marked hypoplastic or aplastic marrow.

What is it that keeps the myeloid tissue functioning at a normal level, keeping the production of neutrophilic cells at a constant pace with their continuous destruction? There are two factors which function in the reproduction, growth, and delivery of the myeloid cells. The first, a maturation factor, causes the original stem cells to multiply and grow to maturity. This may be compared to the hormone which causes the development and ripening of the graafian follicles. When the original stem cells have grown to maturity and the myeloid tissue is formed, the stimulation for further growth is determined by functional demand. This demand in most organs and tissue is brought about by the wearing out of cells; as for example the wearing off of the surface epithelium which constantly stimulates the malpighian layer to activity. This demand, in the case of the myeloid tissue, is furnished in the form of a chemotactic factor which causes the neutrophilic cells to be delivered to the blood stream. As the neutrophilic cells are called to the blood stream, the normal stimulus is to replace them. This normal replacement continues as long as the maturation factor is present, even in long sustained leukocytoses.

Pyogenic organisms exert a chemotactic effect on the myeloid

tissue. All are familiar with the marked sustained leukocytosis in many pyogenic infections. In these cases, the generation of new myelocytes occurs simultaneously with the increased delivery of mature neutrophiles, and the bone-marrow represents a "shift to the left" in the cell phases and an extension of myeloid foci proportionate to the need as long as it exists. This concept of leukocytosis postulates the pyogenic organisms as introducing a chemotactic factor which calls out the cells thereby producing a need for greater production. However, the exact way in which this chemotactic effect is produced is not known. Bacon et al.,[2] consider that this activity of the marrow comes from altered body proteins. It hardly seems likely that these pyogenic organisms introduce this chemotactic factor, but that they introduce a substance which stimulates the body tissues to produce this factor in excess. Some patients having neutropenia have shown a normal neutrophilic response when an infection developed. It is suggested that the organisms stimulated the body tissues to produce the chemotactic factor.

Some organisms exert this chemotactic effect, and at the same time introduce a myeloid or maturation depressant factor. Influenza and typhoid bacilli are examples. The myeloid tissue does not respond with a sustained leukocytosis in these infections, but instead there is a leukopenia. Doan et al.,[11] injected large doses of inactivated typhoid bacilli into animals, and found it was possible to call the neutrophilic cells from the marrow, producing an aplasia of the myeloid tissue without the corresponding activity to replace them. That inactivated typhoid bacilli may have this same depressing effect on the myeloid tissue in susceptible individuals, is indicated by two cases of fatal neutropenia following typhoid vaccination.[5, 21]

The chemotactic effect of nucleic acid and its degradation products (pentose nucleotide, guanine, adenine, et cetera), on the myeloid tissue has been demonstrated by many experiments.[10, 13, 17] It has been shown that the neutrophilic cells, when breaking down in the circulating blood, liberate these products. The theory is that these liberated products in turn stimulate the myeloid tissue to produce more cells.[12, 13, 17, 26] In neutropenia

from any cause, these products would be greatly diminished in the blood stream. Out of this experimental work has come a therapeutic product, nucleotide K-96, made from the nucleic acid of yeast.[18] Doan's[10] experimental work on normal rabbits with nucleotide K-96 would lead to the assumption that it supplies a maturation factor, as well as a chemotactic factor, since, after repeated large doses given to normal rabbits, there was a marked myeloid hyperplasia of the bone-marrow with myeloid deposits in the kidneys and spleen. This can be interpreted as a replacement reaction. When cells are called out from normal marrow, the normal reaction is to replace them. If they are called out in excess, the tendency is toward an over production. In proof of the above interpretation, are the experiments of Harkin[16] who produced neutropenia in rabbits with benzol, and found that pentose neucleotide did not have this effect on the myeloid tissue.

Unfortunately there is much less to be said concerning maturation factors. Our exact knowledge is confined to a maturation factor for erythrocytes. This is supplied in the liver extract and other tissue extracts used in the treatment of pernicious anemia. In pernicious anemia, the erythrocytes cease to multiply and develop properly, development ceasing at the megaloblastic stage, and the erythrocytes in the peripheral circulation are greatly reduced. Liver extract causes the function of normal reproduction to return, which is evidenced by a reticulocyte rise promptly after its use, and the erythropoietic tissue gradually returns to normal. May not malignant neutropenia follow the same course? There are remissions in both pernicious anemia and neutropenia. Perhaps what appear to be fulminating cases of malignant neutropenia with aplastic bone marrow have had many undetected attacks and remissions. These observations point to the fact that there must also be such a growth stimulating substance for the cells of the myeloid tissue. This substance is probably in a remote organ or tissue, as is the maturation factor for erythrocytes. It is not in the spleen, as this function continues normally after splenectomy. The liver seems a likely place for the manufacture of this factor, since this organ functions both as the erythropoietic and granulopoietic organ in early foetal life. The

liver extract now in use does not contain this factor. No doubt there is a specific maturation factor for each stem cell, and it may only require a different process of extraction than that now used.

Based on the theories so far advanced, and the experimental work cited to support them, we may formulate a theory of pathogenesis. Briefly the theory is one of pathological physiology.

Returning to the diagram, we may draw a bracket to embrace the myeloid tissue from the normal level, to and including the hyperplastic. Cases of neutropenia with this pathology can be considered as being due to a decrease or absence of the chemotactic factor. When the chemotactic factor ceases to function, maturation will continue, but the cells will not come to the circulation. This produces a normal and perhaps a hyperplastic myeloid tissue. Another bracket may be drawn embracing the hypoplastic and aplastic marrow. Cases of neutropenia with this pathology can be considered as being due to a decrease or absence of the maturation factor. If the maturation factor ceases to function, the chemotactic factor will continue to call the cells out until an aplasia is produced.

The absence of the chemotactic factor may be just as serious as the absence of a maturation factor, as both result in peripheral neutropenia, and peripheral neutropenia existing for any great length of time, leads to many serious complications and death.

All of the fatal cases so far reported have shown aplastic myeloid tissue, except the five reviewed in this paper. A chemotactic factor was not given to these patients, as nucleotide K-96 was not available at that time. Since the myeloid tissue was hyperplastic, they might have recovered with nucleotide therapy, if it could have been administered before infection was too far advanced.

Patients who recover spontaneously may lack one or the other of these factors, the function resuming without an outside aid, thereby producing a remission.

Biopsies of the bone marrow of Rosenthal's patients who later recovered, indicate that the pathology could have been due to a lack of the chemotactic factor, as the bone marrow was normal or hyperplastic.

There is a cyclic case on record, case 3 of a series reported by

Doan.[9] A neutropenia of an alarming degree developed every twenty-one to twenty-three days. This neutropenia was not influenced by nucleotide K-96, which would tend to indicate that a chemotactic factor was not needed. A report was not made of biopsy of bone-marrow. However, this lack of reaction to nucleotide K-96 would lead to the assumption that the myeloid tissue became markedly hypoplastic every twenty-one days. It would seem that in this patient, the production of the maturation factor functioned in cycles, every twenty-one days ceasing to function for a few days, then resuming functioning.

Jackson et al.,[18] treated a series of thirteen cases of neutropenia with nucleotide K-96. Five of the patients, with no essential clinical differences from those in the recovered cases, died in spite of active nucleotide therapy. Clinically the cases were the same, but evidently the underlying pathology was different. In a larger series, reported at a later date by the same authors,[19] there is practically the same percentage of patients who did not respond to active nucleotide therapy. Does it not seem reasonable to suppose that the recovered cases had normal or hyperplastic myeloid tissue, and lacked a chemotactic factor which was supplied by the nucleotide K-96, while the fatal cases had aplastic myeloid tissue and lacked a maturation factor which could not be supplied? If the myeloid tissue is aplastic, a substance to call the cells to the periphery would be of little value. I believe this same cause was operative in my failure, and the failure of others, to obtain a response of the neutrophils in cases of aplastic anemia and leukanemia treated with neucleotide K-96.

Taussig and Schnoebelin,[27] in a review of 328 cases (which included the so-called secondary types) found that 25 per cent recovered without special therapy and with miscellaneous forms of treatment; 37 per cent recovered with blood transfusion, and 47 per cent with roentgen ray treatment. Nucleotide K-96 has raised the percentage of cures to seventy-four, as the 27 per cent of cases needing a chemotactic factor are now being adequately treated. The 26 per cent now dying in spite of all available treatments may constitute the aplastic, or markedly hypoplastic group, needing a maturation factor.

It is, of course, not contended that anything approximating definite proof of a maturation and chemotactic factor in this disease has been adduced. It does seem reasonable to deduct from the information at hand, that the pathology in the myeloid tissue does not constitute the primary pathologic mechanism of this disease.

This material has been presented in this way to stimulate interest in attacking this problem from a different angle; that is, to search for an organ or tissue extract which will supply a maturation factor for neutrophils. There should be a further search for active principles along the lines of the chemical investigations of Cohn et. al., and West and Howe,[28] a new type of hematological research, in which the goal is to find the chemical substance operative at each stage in the normal processes of division, growth and maturation of neutrophiles. Such a therapeutic agent would aid the cases having hypoplastic and aplastic myeloid tissue, as well as prevent the occurrence of fulminating attacks in the chronic neutropenic patients.

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THE DIFFERENTIATION AND STANDARDIZATION OF CERTAIN STREPTOCOCCUS TOXINS AND ANTITOXINS BY MEANS OF THE SKIN TEST*

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Within the last few years, both in experimental investigations and in practice, the skin test has assumed an important position in the diagnosis of various pathological processes and susceptibilities to many types of infection. Among the earliest uses may be mentioned the Von Pirquet (1907) and the Mantoux (1908) tests in tuberculosis, followed by the Schick Test (1913) for the diagnosis of susceptibility to diphtheria. Skin tests have been used experimentally by Birkhaug (1924) in connection with the description of the hemolytic streptococcus of erysipelas; by Giordano (1929) for the diagnosis of undulant fever; by Ferry, Norton, and Steele, (1931) in experimental studies on meningococcus toxin; and by Thomas and Touart (1932) in conducting a clinical investigation as to the specificity and antigenic response of bacterial antigens. For many years skin tests have been subjected to practical clinical use in the diagnosis of various allergic conditions. These afford but a few illustrations of the scope of the intradermal test.

In 1924, Doctors George F. and Gladys H. Dick announced the results of skin tests with filtrates of blood broth cultures of hemolytic streptococci. This led to the discovery of scarlet fever streptococcus toxin and to the development of the Dick test, now an accepted clinical procedure. For use in the Dick test, scarlet fever streptococcus toxin is standardized as to the number of skin test doses per cubic centimeter, then diluted so that 0.1 cc. contains exactly one skin test dose. This is injected intradermally and the reading is made at the end of twenty-four

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hours. A positive reaction is observed if an area of erythema of 1 cm. or more in diameter surrounds the point of injection. Degrees in susceptibility are determined by the size of the area of erythema as well as by the intensity of the reaction. Through the use of a standardized scarlet fever streptococcus toxin, the official unit of scarlet fever streptococcus antitoxin may be determined. Thus, the unit is defined "in terms of the power to neutralize skin test doses of toxin."

Birkhaug[2] reported that the appearance of positive reaction following the intradermal injection of erysipelas streptococcus toxin is specific and indicates susceptibility to erysipelas. This view has not been shared by Williams,[11] Kirkbride and Wheeler,[6] and others, although Thompson and Thompson[9] in their elaborate review on streptococci state that there can be little doubt that the hemolytic streptococcus of erysipelas "is distinctly different" from that of scarlet fever. Dick and Dick[4] reported the results of studies on specificity of soluble toxins produced by hemolytic streptococci, including the study of twenty-five strains each of hemolytic streptococci isolated from cases of scarlet fever and erysipelas. They called attention to errors in the results of skin tests which may emanate from the use of toxins of insufficient strength, from local tissue immunity, and from sensitivity to serum protein. It was shown by the results of this work that "the soluble toxins produced by scarlet fever and by erysipelas hemolytic streptococci are immunologically specific and distinct." The results of practical clinical use of erysipelas streptococcus antitoxin tend to confirm this conclusion.

It is generally conceded that hemolytic streptococci are frequently associated with puerperal septicemia. Lash and Kaplan[7] followed the work of the Dicks on the hemolytic streptococcus of scarlet fever by publishing the results of their work on a blood broth filtrate of hemolytic streptococcus derived from puerperal septicemia. In subsequent reports, these investigators announced that a true specific toxin was derived from strains of puerperal septicemia streptococci from which antitoxin was produced. The results of skin tests on the same patients with toxins from both scarlet fever and puerperal septicemia strepto-

cocci served to confirm their findings. Williams confirmed the findings of Burt-White,[3] Joe,[5] and Stent,[8] that positive or negative Dick tests were unrelated to pregnancy or to liability to puerperal sepsis. Thompson and Thompson[10] in consideration of the outstanding literature conclude that the majority of severe cases of puerperal septicemia are caused by hemolytic streptococci, but that researches "on toxic filtrates of hemolytic streptococci, from puerperal cases, have so far yielded very little confirmatory evidence in favor of specificity."

Andrewes and Christie[1] found that the most refined methods of agglutination failed to differentiate between some strains of hemolytic streptococci found in scarlet fever, erysipelas, puerperal septicemia. They concluded that perhaps the important criterion in identifying these organisms depended upon the degree of intensity or potency of specific toxins which they were able to produce. Therefore, it is important that reliable data be accumulated bearing upon the specificity of the toxins from the scarlet fever and erysipelas hemolytic streptococci, and especially upon that derived from hemolytic streptococci found in puerperal septicemia.

PRESENT INVESTIGATION

During the last few years, an opportunity has been afforded the writers to study the results of skin tests involving the use of a relatively large number of samples of toxins and antitoxins derived from the scarlet fever and erysipelas hemolytic streptococci, and hemolytic streptococci isolated from severe cases of puerperal sepsis.

Since 1928, 6959 human subjects, including those tested for susceptibility, have been employed, involving 27,713 individual intradermal injections, in determining the potencies of many different lots of the respective toxins and antitoxins. Records for the past four years include the following: scarlet fever streptococcus toxins and antitoxins, 2390 susceptible human subjects, involving 15,528 individual intradermal injections; erysipelas streptococcus toxins and antitoxins, 336 susceptible human subjects, involving 1654 individual tests; puerperal septi-

cemia streptococcus toxins and antitoxins, 160 susceptible human subjects, involving 811 individual tests. The above total number of susceptible human subjects does not refer to individual but rather to test subjects. In many cases the same individuals were subjected to repeated tests.

This experience serves as a background for the present investigation, the purpose of which is (1) to show the significance of cross reactions among human susceptibles to the toxins of scarlet fever, erysipelas, and puerperal septicemia hemolytic streptococci, respectively; (2) to show the degree of specificity of the respective antitoxins toward the homologous toxins.

TOXINS EMPLOYED, TECHNIQUE, AND NATURE OF TESTS

Government standard scarlet fever streptococcus toxin has been used throughout this work. No officially recognized standardized toxin exists with respect to the erysipelas hemolytic streptococcus or to hemolytic streptococci obtained from puerperal septicemia. As has been pointed out by the Dicks, much confusion has resulted in work previously reported because of the lack of standardization of toxins used in conducting skin tests. They found that the filtrate from erysipelas strains contains considerably weaker toxin than that from the scarlet fever streptococcus. It is observed that the same condition obtains in toxins derived from the hemolytic streptococci found in puerperal septicemia. Relatively few strains are found which will produce toxin possessing a potency of more than 2500 skin test doses per cubic centimeter. No toxins should be employed containing less than 2500 skin test doses per cubic centimeter, because if the final dilution in the neutralization test is less than 1:250, nonspecific protein reactions from the culture medium may be mistaken for positive skin reactions. The government standard scarlet fever streptococcus toxin which has been used throughout contains 4500 skin test doses per cubic centimeter. The erysipelas streptococcus toxin used in the early part of this work was derived from three strains, one of which was received from Dr. Sanford, Mayo Clinic, and two of which were isolated from blood cultures at the Henry Ford and Herman Kiefer Hospitals,

TABLE 1

Scarlet fever streptococcus antitoxin	90 lots
Scarlet fever streptococcus antitoxin	884 horse serum samples
Scarlet fever streptococcus toxin	81 lots
Erysipelas streptococcus antitoxin	49 lots
Erysipelas streptococcus antitoxin	82 horse serum samples
Erysipelas streptococcus toxin	24 lots
Puerperal septicemia streptococcus antitoxin	19 lots
Puerperal septicemia streptococcus antitoxin	51 horse serum samples
Puerperal septicemia streptococcus toxin	17 lots

TABLE 2

ERYSIPELAS STREPTOCOCCUS ANTITOXIN (SERUM SAMPLES)

SUBJECT	READING	TEST* TOXIN 1 S.T.D.	SERUM† SAMPLE 3025	SERUM SAMPLE 3027	SERUM SAMPLE 3035	SERUM SAMPLE 3101	SERUM SAMPLE 3159	SERUM CONTROL
	hours							
1	24	25x30 R‡	12x13 R	9x 9 R	9x 9 R	12x11 R	7x 7 R	Neg.
	48	25x30 FR	12x12 R	9x11 R	12x12 R	11x12 R	11x11 R	Neg.
2	24	Serum reaction						
	48	Serum reaction						
3	24	20x20 R	10x11 R	9x 8 R	9x 9 R	11x11 R	7x 7 R	Neg.
	48	20x20 FR	11x11 R	11x11 R	11x15 R	11x12 R	8x 9 R	Neg.
4	24	25x20 R	12x11 R	11x11 R	12x12 R	11x12 R	12x12 R	Neg.
	48	20x20 R	12x11 R	11x11 R	12x12 R	11x12 R	8x 9 R	Neg.
5	24	25x25 R	12x12 R	10x10 R	9x 9 R	11x12 R	8x 9 R	Neg.
	48	25x25 R	12x11 R	10x10 R	13x13 R	12x13 R	11x12 R	Neg.
Values§			3 plus	3 plus	2 plus	3 plus	3 plus	

* One-tenth cubic centimeter (1:300 dilution) or one skin test dose toxin.

† Serum samples from individual horses under immunizing treatment mixed with one skin test dose toxin.

‡ R = red. FR = faint red.

§ Determined on 48-hour readings as follows: 4 plus strong = complete neutralization at 100 units. 4 plus = neutralization (area of erythema less than 1.0 cm. in all subjects). 3 plus = reaction from 1.0 cm. to 1.3 cm. 2 plus = reaction from 1.3 cm. to 1.5 cm. 1 plus = reaction from 1.5 cm. to 1.8 cm. N.G. = reaction above 1.8 cm.

Detroit. This toxin has been found to contain 3000 skin test doses per cubic centimeter, the dilution of the toxin used as the

standard in the tests being 1:300. The original test toxin derived from the bouillon filtrates of streptococci from puerperal septicemia cases represented three strains obtained from blood cultures. The toxin resulting from the growth of these cultures has been found to contain 4000 skin test doses per cubic centimeter, requiring for 0.1 cc. intradermal injection a dilution of 1:400. A new test toxin has been prepared from the same strains grown under different conditions, which produces more distinct reactions and probably contains slightly more than

TABLE 3

PUERPERAL SEPTICEMIA ANTITOXIN (SERUM SAMPLES)

SUBJECT	READING	TEST TOXIN 1 S.T.D.	SERUM SAMPLE 3092	SERUM SAMPLE 3113	SERUM SAMPLE 3114	SERUM CONTROL
	hours					
1	24	20x23 R	Neg.	10x13 R	10x10 R	Neg.
	48	20x23 FR	Neg.	12x13 R	11x12 R	Neg.
2	24	20x20 R	Serum reaction			
	48	20x22 R	Serum reaction			
3	24	18x18 R	10x10 R	11x11 R	10x11 R	Neg.
	48	18x18 R	10x 9 R	12x12 R	10x10 R	Neg.
4	24		Unable to obtain reading			
	48	25x30 R	7x 7 R	13x14 R	11x11 R	Neg.
5	24		Unable to obtain reading			
	48	18x20 R	7x 8 R	13x14 R	11x11 R	Neg.
Values			4 plus	2 plus	3 plus	

4000 skin test doses per cubic centimeter. The above toxins have been used in the routine testing of the lots of materials listed in table 1.

The technique employed has been that which is followed routinely in observing the requirements of the National Institute of Health and of the Scarlet Fever Committee with respect to the standardization of scarlet fever streptococcus antitoxins and toxins.

Protocols are submitted as illustrative of the nature of the

intradermal tests on various lots of serum samples of erysipelas, and puerperal septicemia streptococcus antitoxins in tables 2 and 3.

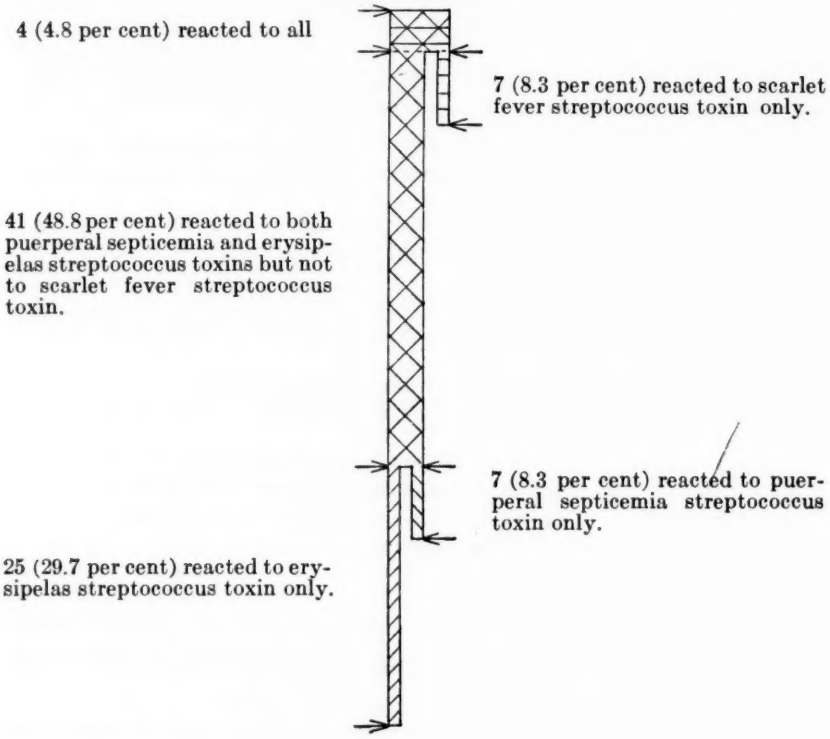
CHART 1

CROSS REACTIONS

Out of 221 individuals tested for susceptibility to scarlet fever, puerperal septicemia and erysipelas streptococcus toxins,

137 (62 per cent) reacted to none	84 (38 per cent) reacted to one or more

The 84 reactors were distributed as follows:



EXPERIMENTAL DATA

In order to determine the extent of cross reactions which might be observed among human subjects susceptible to one or more of the three toxins under consideration, a group of 221 subjects was

selected who were tested each with one skin test dose intradermally of the three toxins. One hundred and thirty-seven or sixty-two per cent reacted to none of the three toxins, while eighty-four, or 38 per cent, reacted to one or more. Of the total original 221 subjects, positive reactions were observed as follows: to scarlet fever, eleven, or 5 per cent; to erysipelas, seventy, or 31.6 per cent; to puerperal septicemia, fifty-three, or 23.5 per cent. Of the eighty-four reactors, four (4.8 per cent) individuals reacted to all three toxins; seven (8.3 per cent) to scarlet fever streptococcus toxin only; twenty-five (29.7 per cent) to erysipelas streptococcus toxin only; seven (8.3 per cent) to puerperal septicemia streptococcus toxin only; and forty-one (48.8 per cent) to both erysipelas and puerperal septicemia streptococcus toxins. The results are shown in chart 1.

In the above series of reactors were found a certain proportion of individuals who were susceptible to only one of three toxins in each case. Of eighty-four reactors to one or more of three types of hemolytic streptococcus toxins, the following observations were made:

(1) Of the individuals positive to scarlet fever, 36.4 per cent were positive to erysipelas.

(2) Of the individuals positive to scarlet fever, 36.4 per cent were positive to puerperal septicemia.

(Those positive to erysipelas were also positive to puerperal septicemia and represent identical subjects, only four individuals being involved.)

(3) Of the individuals positive to erysipelas, 5.9 per cent were positive to scarlet fever.

(4) Of the individuals positive to erysipelas, 64.3 per cent were positive to puerperal septicemia.

(5) Of the individuals positive to puerperal septicemia, 7.7 per cent were positive to scarlet fever.

(6) Of the individuals positive to puerperal septicemia, 86.5 per cent were positive to erysipelas.

No evidence is observed which shows any direct interrelation between the toxins of the three hemolytic streptococci.

In order to study further the possibility of cross neutralization

TABLE 4

SPECIFICITY OF PUERPERAL SEPTICEMIA STREPTOCOCCUS TOXIN AGAINST NEUTRAL MIXTURES OF PUERPERAL SEPTICEMIA, ERYSIPELAS, AND SCARLET FEVER STREPTOCOCCUS ANTITOXINS

SUBJECT	READING	TEST TOXIN PUERPERAL SEPTICEMIA 1:400 DILUTION 0.1 CC.	PUERPERAL SEPTICEMIA TOXIN PLUS PUERPERAL SEPTICEMIA ANTITOXIN	PUERPERAL SEPTICEMIA TOXIN PLUS ERYSIPELAS ANTITOXIN	PUERPERAL SEPTICEMIA TOXIN PLUS SCARLET FEVER ANTITOXIN	SERUM CONTROL	PROTEIN (BROTH AND BLOOD) CONTROL
	hours						
1	24	No reaction					
	48	No reaction					
2	24	15x18 R	Neg.	15x15 R	15x16 R	Neg.	Neg.
	48	15x17 R	Neg.	15x15 R	15x15 R	Neg.	Neg.
3	24	20x21 R	7x 6 R	20x20 R	20x19 R	Neg.	Neg.
	48	20x20 R	10x11 R	20x18 R	19x19 R	Neg.	Neg.
4	24	15x20 R	Neg.	12x13 R	14x15 R	Neg.	Neg.
	48	18x23 R	Neg.	13x13 R	14x13 R	Neg.	Neg.
5	24	15x15 FR	Neg.	14x14 R	14x14 R	Neg.	Neg.
	48	15x15 FR	Neg.	16x15 R	16x16 R	Neg.	Neg.
6	24	15x17 R	Neg.	Neg.	Neg.	Neg.	Neg.
	48	15x15FR	Neg.	Neg.	Neg.	Neg.	Neg.

TABLE 5

SPECIFICITY OF ERYSIPELAS STREPTOCOCCUS TOXIN AGAINST NEUTRAL MIXTURES OF SCARLET FEVER, ERYSIPELAS, AND PUERPERAL SEPTICEMIA STREPTOCOCCUS ANTITOXINS

SUBJECT	READING	TEST TOXIN ERYSIPELAS 1:300 DILUTION 0.1 CC.	ERYSIPELAS TOXIN PLUS ERYSIPELAS ANTITOXIN	ERYSIPELAS TOXIN PLUS PUERPERAL SEPTICEMIA ANTITOXIN	ERYSIPELAS TOXIN PLUS SCARLET FEVER ANTITOXIN	SERUM CONTROL	PROTEIN (BROTH AND BLOOD) CONTROL
	hours						
1	24	17x20 R	Neg.	14x16 R	17x20 R	Neg.	Neg.
	48	17x22 R	Neg.	14x17 R	17x20 R	Neg.	Neg.
2	24	20x23 R	Serum reaction			25x29 R	Neg.
	48	20x23 R	Serum reaction			25x30 R	Neg.
3	24	20x20 R	Serum and protein reaction			Diffuse	Diffuse
	48	20x22 R	Serum and protein reaction			Diffuse	Diffuse
4	24	22x25 R	7x7 R	20x22 R	20x25 R	Neg.	Neg.
	48	22x27 R	10x12 R	22x25 R	23x27 R	Neg.	Neg.
5	24	24x20 R	Serum reaction			20x20 R	Neg.
	48	22x22 R	Serum reaction			22x25 R	Neg.

and the possible inter-relationship between those reacting to the toxins of the scarlet fever, erysipelas, and puerperal septicemia streptococci, neutralization tests with the various antitoxic serums were conducted. Six human subjects, who had reacted positively to one skin test dose of puerperal septicemia streptococcus toxin, were tested with mixtures of the specific test toxin,

TABLE 6

SPECIFICITY OF SCARLET FEVER STREPTOCOCCUS TOXIN AGAINST NEUTRAL MIXTURES OF SCARLET FEVER, ERYSIPELAS, AND PUERPERAL SEPTICEMIA STREPTOCOCCUS ANTITOXINS

SUBJECT	READING	GOVERNMENT STANDARD SCARLET FEVER TOXIN 1:4500 DILUTION 0.1 CC.	STANDARD SCARLET FEVER TOXIN 1:400 DILUTION PLUS SCARLET FEVER STANDARD ANTITOXIN	SCARLET FEVER TOXIN PLUS ERYSIPELAS ANTITOXIN	SCARLET FEVER TOXIN PLUS PUERPERAL SEPTICEMIA ANTITOXIN	SERUM CONTROL	PROTEIN (BROTH AND BLOOD) CONTROL
	hours						
1	24	20x22 R	Neg.	19x20 R	20x20 R	Neg.	Neg.
	48	20x22 R	Neg.	19x22 R	22x22 R	Neg.	Neg.
2	24	15x18 R	Serum reaction, diffuse				Neg.
	48	17x18 R	Serum reaction, diffuse				Neg.
3	24	20x21 R	Neg.	20x21 R	20x23 R	Neg.	Neg.
	48	20x20 R	Neg.	19x21 R	20x23 R	Neg.	Neg.
4	24	No reaction					
	48	No reaction					
5	24	17x16 R	Neg.	14x16 R	14x14 R	Neg.	Neg.
	48	17x16 FR	Neg.	15x17 R	16x17 R	Neg.	Neg.
6	24	15x15 R	Neg.	15x15 R	14x12 R	Neg.	Neg.
	48	17x15 R	Neg.	17x17 R	16x17 R	Neg.	Neg.

puerperal septicemia streptococcus antitoxin, erysipelas streptococcus antitoxin, and scarlet fever streptococcus antitoxin, respectively. Similar tests were conducted on five individuals susceptible to the toxin of the erysipelas streptococcus, and also on six positive Dick reactors. The results are shown in tables 4, 5 and 6.

Among six human subjects initially susceptible to the toxin of puerperal septicemia hemolytic streptococcus, one failed to react to the toxin. In the remaining five, complete neutralization of puerperal septicemia toxin by homologous antitoxin was observed in all but one, but no neutralization occurred with the antitoxins from scarlet fever and erysipelas streptococci. Likewise in the group of five previously found to react to erysipelas streptococcus toxin, two reactors showed satisfactory neutralization with homologous antitoxin, but failed to show neutralization to puerperal septicemia and scarlet fever streptococcus antitoxins, while none of four satisfactorily positive Dick reactors showed evidence of cross neutralization to erysipelas and puerperal septicemia antitoxins. It is recognized that at the present time scarlet fever streptococcus toxin is relatively stronger than either erysipelas or puerperal streptococcus toxins, which fact perhaps serves to explain the slight tendency toward cross neutralization in one instance in each series (erysipelas, and puerperal septicemia). The experimental evidence, however, definitely points toward specificity.

SUMMARY

The results of the above experimental work not only confirm the conclusions which were reached by the Dicks that "the soluble toxins produced by scarlet fever and erysipelas streptococci are immunologically specific and distinct" but suggest furthermore that the toxin produced by certain hemolytic streptococci isolated from cases of puerperal septicemia is specific and distinct.

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HISTOLOGY AFTER THORIUM DIOXIDE (THOROTRAST) IN HEPATOLIENOGRAPHY*

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The advent of a method of roentgenographic visualization of the liver and spleen in the human has opened a vast new field of scientific investigation. The procedure is based upon a physiologic principle of the reticulo-endothelial cells; that is, the characteristic property of engulfing colloidal particles which may be introduced into the circulation. That the method represents a definite step forward in hepatolienography has been confirmed many times. Yet, there has been considerable conjecture concerning many of the phases, as for example, the radioactivity of the metal; the hemoclastic activity of the colloidal suspensions; and the permanence of the thorium particles in the reticulo-endothelial cells with possible toxic effects produced after a long period of time.

Since the last clinical paper[25] published by myself and associates numerous results have been reported by various authors concerning different phases of the problem. We have continued our work, reporting [4, 24, 26] results from time to time. An attempt has been made to check these results with the work of other investigators; in each instance, sufficient experiments in a number of animals being performed in order to reduce to a minimum results which may be accidental. It is well known to those who do a great deal of work with animals that in isolated instances, many phenomena occur which are purely accidental or merely coincidental. Certainly, proper interpretation of results in the light of these facts is essential before any conclusions may be drawn.

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For a period of over two years, dogs, rabbits, guinea pigs, white mice and albino rats were injected with various dosages of thorotrast. The number totalled well over one hundred animals, with controls in every experiment. Later patients in whom it was deemed essential that further information regarding the liver and spleen be obtained for correct diagnosis were injected for diagnostic purposes. In all instances clinical data were noted in conjunction with clinicopathologic and histopathologic studies which were made at every opportunity.

It is the purpose of this paper to note these observations and attempt to correlate the findings with those of other investigators, in order to thoroughly understand the principles of the method, its possibilities, limitations and probable contraindications.

HISTORY

In 1929, M. Oka,[15] while studying the metabolism of thorium dioxide in rabbits, noted that upon its administration only a fraction of the injected thorium was eliminated. He took radiographs of various parts of the body (thorium dioxide being a heavy metal, is opaque to the roentgen ray) and found shadows conforming in position to the spleen. He reported his finding, but did not explain the mechanism by which these organs could be made visible roentgenographically.

Immediately following Oka's publication, P. Radt[18] and Jaffé[8] began their work showing the thorium particles were engulfed from the circulation by the reticulo-endothelial cells, thus rendering the organs in which these particular cells happen to be present more opaque to the roentgen ray. The thorium dioxide used was marketed under the name of tordiol, which was toxic due to the low dispersion factor of the solution and flocculation properties when brought into contact with organic matter. Their work was repeated by Oka.[16] Later Radt[19, 20] succeeded in preparing the thorium dioxide in a so-called "stabilized state," in which it would not flocculate when brought into contact with body fluids and in increasing the degree of dispersion by relatively high concentrations of glucose solutions. This final substance is now marketed under the name of "thorotrast-Heyden."

The solution used contains 25 per cent thorium dioxide by volume suspended in a stabilized colloidal state. The element, thorium, is opaque to the roentgen rays, being a heavy metal (atomic weight, 232.15). On administration into the blood stream, the reticulo-endothelial cells engulf the particles of thorium dioxide. Since these cells are present in relatively large numbers in the spleen and liver, there is a difference in shadows cast by these organs and the surrounding tissues that contain few, if any, reticulo-endothelial cells. Whereas, before the introduction of this method, only faint rather indefinite outlines of the liver and spleen were obtainable by roentgenography, it is now possible not only to study the size and shape of these organs, but to determine more accurately whether any tumor, cyst or abscess is present in these organs or whether a certain tumor in the hepatic or splenic regions is within or outside the liver or splenic substances.

The question of dosage is of paramount importance. Kadrnka[9] found that not more than 0.8 cc. of thorotrast per kilogram of body weight was required in the human to produce good contrast between the shadows cast by these organs and the surrounding soft tissue. Satisfactory radiographs of the liver and spleen have been obtained, using this dosage, in both our experimental animals and in the human.

The work was then taken up in this country by Yater and Otell,[28] Stewart, Einhorn and Illick[23] and my coworkers.[25]

The reticulo-endothelial cells form the component parts or units of a system designated by Aschoff[1] as the "reticulo-endothelial system." The cells of this group are widely scattered throughout the body, being collected in relatively large numbers in the spleen, liver, medullary follicles and "cords" of lymph nodes. Considerable numbers are also found in the formative bone marrow, lung and in the adrenal and pituitary bodies. Maximow[14] refers to the individual cells as "histiocytes" and cites sufficient reasons to bear out his contention for this nomenclature. These cells have the characteristic property of engulfing colloidal particles in fine granular form which may be present in the circulating body fluids. It is this definite functional

capacity which distinguishes this type of cell from the connective tissue elements and from all forms of myeloid and lymphatic cells. In this connection, however, it is well to recall that practically any type of cell may become phagocytic if suitably and sufficiently stimulated. Only those cells which have a native avidity for colloidal particles must be included in the reticulo-endothelial system. For instance, cells which become pigment-laden only after dye particles are injected in large amounts and in great concentration are not necessarily of the so-called reticulo-endothelial type.

The "endothelial" part of the term "reticulo-endothelial system" is derived from the fact that these cells form an interrupted layer lining many of the sinuses of several organs, namely, the liver and spleen. The question arises concerning the relationship of the reticular fibrils with the "reticulo" part of the term. Corner,[3] confirming the work of Mall,[13] has demonstrated the presence of extremely fine intracellular fibrils. These cytoplasmic projections branch out from the cells and form a fine reticular network in the intercellular spaces. As a result of this work, there exists another criterion, morphology, by which is distinguished the basic cells of the so-called "reticulo-endothelial system."

Numerous investigators have studied the problem in its various phases and from their studies results have been obtained and conclusions drawn, which are in many instances directly divergent. Radt[18, 19, 20] and Kadrnka[9, 10] after considerable experimentation in animals from a clinical and histopathologic standpoint obtained results from which they concluded that the method is most valuable as a clinical aid and quite harmless in the dosage used. Indeed, in certain of Kadrnka's cases, a definite beneficial therapeutic effect was obtained, although sufficient clinical data had not been accumulated to draw any sweeping conclusions. Otell[17] after thoroughly reviewing the subject in both experimental animals and in the human, could not definitely demonstrate depression of the function of the cells comprising the reticulo-endothelial system. Yater and Otell[29] conclude from a study of about eighty cases that, "It (Thorotrast)

is apparently harmless and contraindications are negligible. Reactions are few and are not serious." Yater[30] has used thorotrast in one hundred human subjects with satisfaction. Lewisohn[12] following the utlization of thorotrast in four rabbits and six patients, concluded that, "the intravenous injection of Thorotrast in quantities mentioned has no immediate ill-effect on the patient." In this latter series the actual dosage was only noted in one case and the amount injected was 60 cc. (five doses of 12 cc. each). It is presumed that all patients received the same dosage.

The effect following intravenous thorotrast upon the immune mechanism has been studied by Held[6, 7]. From the results obtained in his experiments with rabbits, he assumed that in the human, after the thorium dioxide particles have been completely stored by the reticulo-endothelial cells, no damage to this means of protection against infection need be feared. He also showed that only slight influence was exerted by thorotrast on the formation of antibodies; explaining his results by the fact that only a small part of the tissue concerned in the formation of antibodies stores the thorotrast.

However, other workers have reported results from which opposite conclusions have been drawn. Stewart, Einhorn and Illick[23] reported eight cases, including one in which a fatal hemorrhage occurred from a carcinoma of the stomach following thorotrast injections. They also found evidence of radioactivity in splenic tissue removed at autopsy from a patient following thorotrast injection. When the splenic tissue was placed in a petri dish in contact with a photographic plate, evidence of activity on the plate was noted after one day. Harris and Friedrichs[5] working with white rats in which amounts of thorium dioxide varying from 0.2 cc. to 1 cc. was injected reported severe changes in the liver and spleen. Shih and Jung[21] reported work with rabbits in which thorotrast was used in doses varying from 1.0 cc. to 9.0 cc. per kilogram. They reported,

> All rabbits displayed at autopsy extensive extravasations of blood in various internal organs. It is concluded that thorotrast, probably due to its content of thorium dioxide given intravenously in rabbits, tends to lower the thrombocytic

content of the blood and to produce acute purpura hemorrhagica; amounts of three to four times the standard dose are usually fatal. The fact that the standard doses were given in two, rather than in four fractions as given by Kadrnka, may well account for the difference in our results and his.

Shute and Davis[22] have recently reviewed the literature in a most complete manner. By using dosages of approximately 6 cc. to 7 cc. per kilogram of body weight in dogs and rabbits, they were not successful in attempts at roentgenographic visualization of the placenta of these pregnant animals as reported by previous investigators. They noted intense degeneration, particularly in the liver and spleen. One of their rabbits aborted and a spontaneous rupture of the spleen with hemorrhage was found in this animal.

Whitaker, Davie and Margatroyd[27] recently tabulated the advantages and disadvantages of the method and described the histologic and histopathologic pictures in the spleen, bone marrow, lung, kidney and suprarenal gland of rabbits. The rabbits were given one or two doses of thorotrast. The dosage was roughly proportionate to the maximum of that recommended for human beings.

EXPERIMENTAL METHODS

In the light of this maze of conflicting experimental and clinical results, sufficient experiments were performed in various animals using dosages of thorotrast varying from that which produced satisfactory roentgenographic visualization of the liver and spleen (0.8 cc. per kilogram of body weight), to ten times this dosage (8.0 cc. per kilogram of body weight). The complete dose was divided into three fractions, each being given twenty-four hours apart. Observations of the internal organs were made at varying times by means of laparotomies and postmortem examinations. Practically all tissues of the body were taken at autopsy and sections of the spleen and liver taken at operation. The time at which the sections were taken following thorotrast injection varied from twenty-four hours to two years and two months.

Eighty-six animals were used in this work, including five dogs, fifteen albino rats, eighteen guinea pigs, twenty-one white mice

and twenty-seven rabbits. An additional number of animals were used as controls in each experiment. In addition, histologic studies were made at necropsy of the patients who were injected for diagnostic purposes.

A. Animals receiving 0.8 cc. per kilogram of body weight divided into three doses, each dose given twenty-four hours apart

1. *Liver.* At the end of twenty-four hours a few thorium particles were still found free in the capillaries and in the liver cells. However, the greater portion

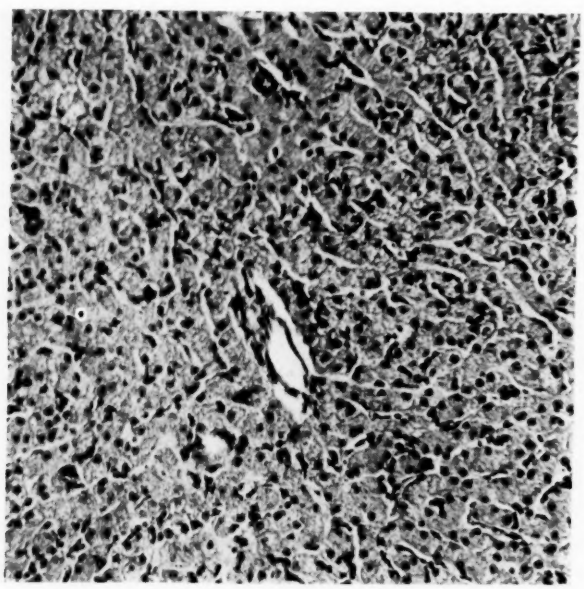

FIG. 1. LIVER OF DOG

One month following 0.8 cc. thorotrast per kilogram body weight. Reticulo-endothelial cells filled with thorium dioxide particles. Liver cells do not show any remarkable changes. × 350

had already been engulfed by the reticulo-endothelial cells, which had become considerably larger in size. At the end of one week no free thorium was found, but the reticulo-endothelial cells had become swollen and the nuclei had begun to take a position near the periphery of the cell. Only an occasional thorium granule was present in the liver cells. One month after the injection of the thorium, no particles were seen in the liver cells, but a considerable amount was still present in the reticulo-endothelial cells diffusely throughout the organ (figs.

1 and 2). A few of the latter cells were noted in which the nuclei had divided and migrated to the periphery of the cell. Six months later practically the same picture was presented, except that here and there a few reticulo-endothelial cells not containing the thorium dioxide particles were found. At the end of two years and two months the thorium particles were still present in considerable amounts. The reticulo-endothelial cells containing the particles were no longer diffusely distributed throughout the organ, but had become aggregated in areas here and there. The thorium particles had apparently become considerably larger in size, entirely filling the cells. The nuclei of the reticulo-endothelial cells had divided and migrated to the periphery of the cell. These cells had

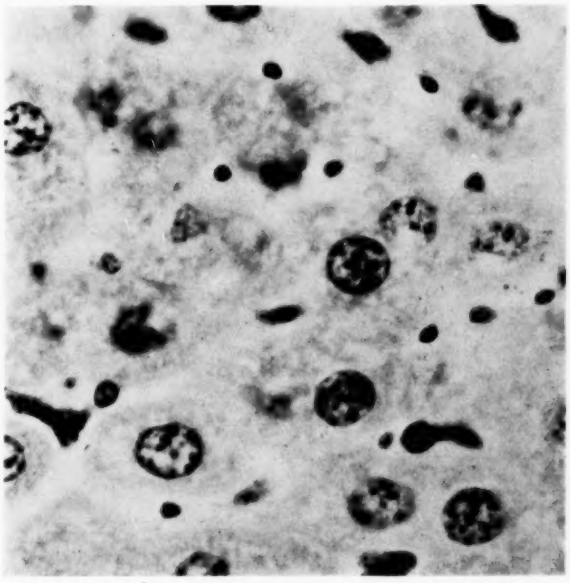

FIG. 2. SAME AS FIG. 1. × 500

become as large or slightly larger than the liver cells and had aggregated in the sinuses as to almost completely fill their lumens. So packed were the thorium particles in the cells that the cytoplasm was no longer visible. Despite this accumulation of the thorium particles into some of the cells, other reticulo-endothelial cells showed no particles in them (fig. 8). Apparently, the reticulo-endothelial cells were dividing, the younger cells taking up the thorium particles which were liberated by the older cells which had succumbed. Those containing the thorium seemed to have a greater avidity for more particles, possibly due to the selectivity of different reticulo-endothelial cells for different substances. When one substance is present in a particular cell, it seems that as more of that

certain substance becomes available, the cell continues to engulf it. No evidence of hemorrhage or fibrosis could be determined in any of the sections studied.

2. *Spleen.* As regards the free particles of thorium and the reticulo-endothelial cells, the spleen presented essentially the same picture as seen in the liver at the various time intervals. In addition, there also appeared a few large giant cells, not of the multinucleated foreign body type, in tissue sections of

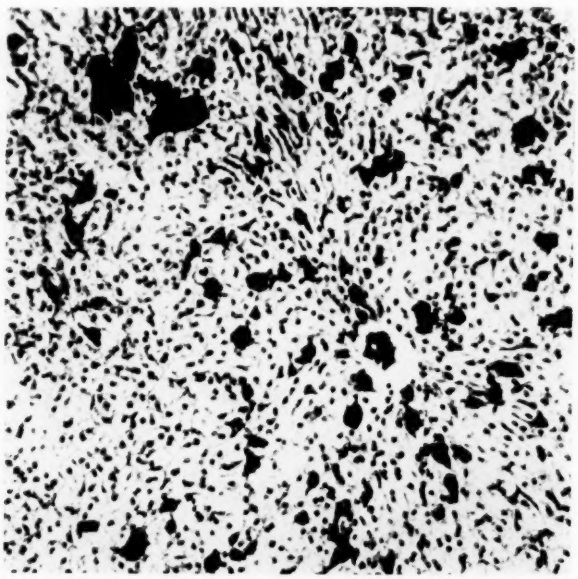

FIG. 3. LIVER OF RABBIT

One day following 8.0 cc. thorotrast per kilogram body weight. Thrombi of thorium particles in sinuses and extensive degeneration of liver tissue. × 350

animals six months after injection. In the tissues removed at the end of two years, these cells were present in considerable numbers throughout the section. At no time did they manifest any phagocytic propensities (fig. 9). No evidence of hemorrhage or fibrosis or necrosis could be determined in any of the sections studied.

3. As a rule, none of the other organs revealed any evidence of thorium particles except the lungs. The later sections of lung showed the presence of a greater number of thorium-filled reticulo-endothelial cells than those tissues examined in the earlier periods. This was probably due to the migration of these cells to the lung, this organ being one of the routes of excretion.

B. Animals receiving 8.0 cc. thorotrast per kilogram of body weight divided into three doses, each dose being given twenty-four hours apart

1. *Liver.* Histological studies one day after injection revealed numerous thrombi present in the sinuses. On closer examination the reticulo-endothelial cells were literally loaded with the thorium granules and a great deal of thorium was found free in the sinuses and between the cells. A considerable number of thorium particles were found in the liver cells and occasionally a free thorium dioxide granule here and there in the bile ducts. There was an intense paren-

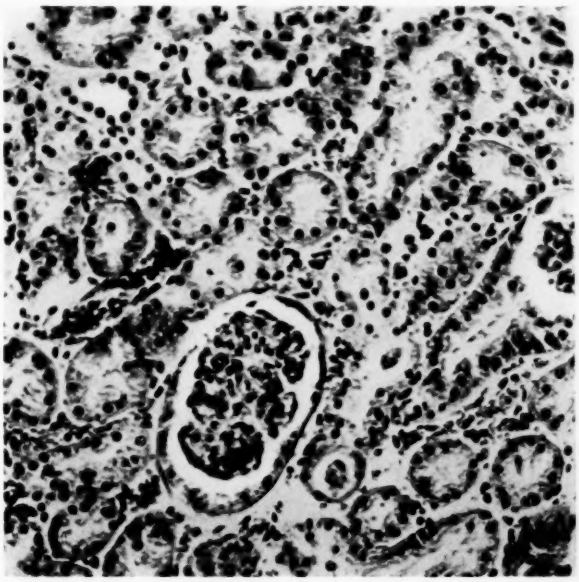

FIG. 4. KIDNEY OF GUINEA-PIG

One week following 8.0 cc. thorotrast per kilogram body weight. Glomerular capsule swollen and cloudy swelling of tubules present. × 400

chymatous degeneration of the liver cells, many of which showed the nuclei to be pyknotic. Only slight, if any, actual necrosis of cell protoplasm was found (fig. 3). At the end of one week practically all of the thorium was seen within the reticulo-endothelial cells. There was a notable increase in the number of these cells. Coincidentally, many of the nuclei of these cells showed pyknosis and fragmentation. A few of the nuclei of the liver cells showed mitosis; probably evidence of regeneration of liver parenchyma. At the end of six months, much of the thorium had disappeared and the number of the reticulo-endothelial cells containing the thorium had become slightly lessened. However, the cells were markedly enlarged and formed thrombi in the venous sinuses. Some tho-

rium was found free, probably due to fragmentation of the reticulo-endothelial cells which had succumbed. The liver cells did not show any thorium particles present, but many of the nuclei had migrated to the periphery of the cell. No hemorrhage or fibrosis was noted in this organ.

2. *Spleen.* The changes seen here were similar to those observed in the liver at the various stages, there being innumerable thrombi of thorium particles both free and in the enormously enlarged reticulo-endothelial cells. Here and there, minute hemorrhages with beginning necrosis resulting from capillary

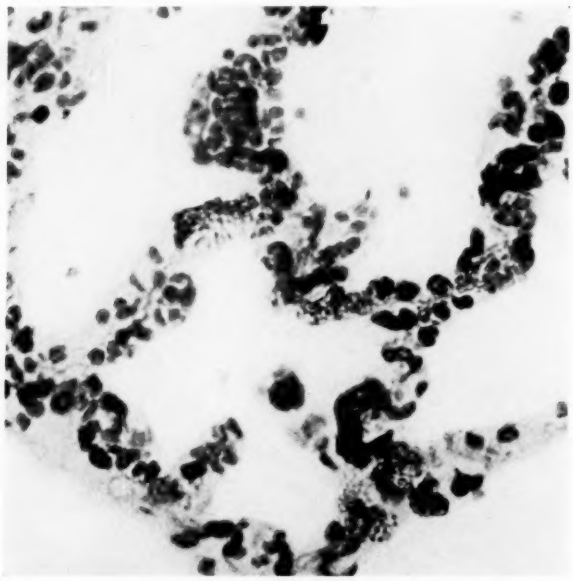

FIG. 5. LUNG OF WHITE RAT

One month following 8.0 cc. thorotrast per kilogram body weight. Reticulo-endothelial cells contain many thorium dioxide particles. × 400

thrombosis was noted. At the end of one week, a most interesting histological picture from the standpoint of distribution of the reticulo-endothelial cells was observed in the spleens of the various animals. The spleens of the guinea pigs and rabbits revealed the thorium particles in the cells at the center of the Malpighian bodies, whereas the same organ in the white rats and mice presented the thorium particles in the cells at the periphery of the Malpighian body. At the end of one month and later, the only changes in the picture was a diminution in the amount of thorium present in the organ, the histological changes lasting throughout the period of observation.

3. *Kidneys.* Those animals sacrificed at the end of one day and one week

showed exudate with thickening of the glomerular capsule and thrombi of thorium in the capillary tufts.. Considerable granular degeneration of the proximal convoluted tubules was found. However, at the end of one month and later, these changes were no longer found (fig. 4).

4. Thorium particles were found in the reticulo-endothelial cells of bone marrow (fig. 6), adrenal glands and a considerable amount in the lung (fig. 5). A few of the reticulo-endothelial cells filled with thorium were found in the lumen of the alveoli of the lung. No further notable changes in histological structure

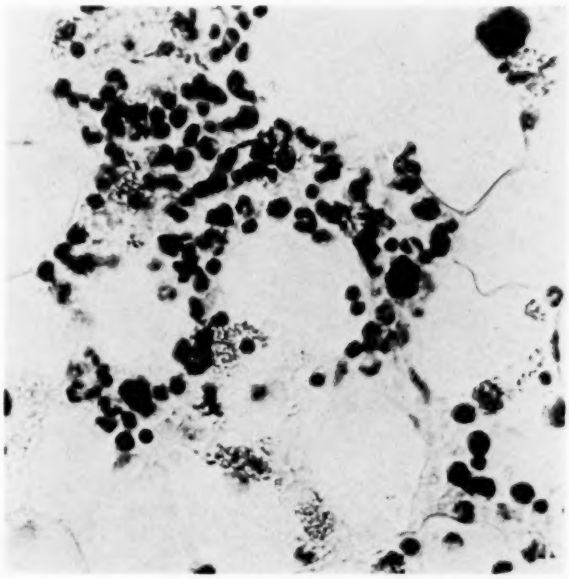

FIG. 6. BONE MARROW OF DOG

One week following 8.0 cc. thorotrast per kilogram body weight. Numerous reticulo-endothelial cells are seen containing thorium dioxide granules. × 400

other than the presence of thorium particles were seen in any of these tissues studied.

5. Except in rare instances, no notable number of thorium particles were found in the sections examined from the brain, heart muscle, pancreas, genital organs, lymph nodes, or tissues of the embryos of the pregnant animals. Those animals wherein the intracardiac administration of the thorium was practised, accidental injection of the mediastinal tissues resulted in thorium particles being present in the pericardial and pleural sacs and also in the draining lymph nodes. Only a few of the animals were killed by punctures of the pleural sacs following laparotomy, while under the influence of chloralose, during experiments con-

cerning splenic contraction.[4] The great majority were sacrificed by sudden dislocation of the skull from the vertebral column. All control animals were sacrificed by respective methods.

CLINICAL AND HISTOLOGICAL RESULTS IN HUMAN CASES

Approximately twenty-five patients in whom it was deemed essential that further information regarding the liver and spleen

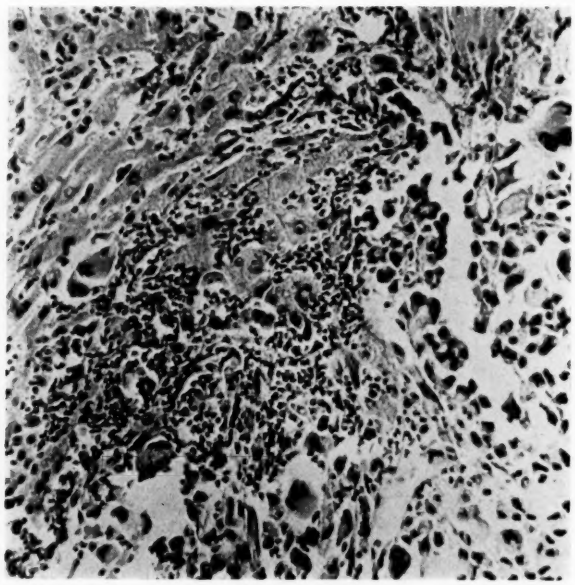

FIG. 7. LIVER, HUMAN

Forty-eight hours after 0.8 cc. thorotrast per kilogram body weight. Section at edge of metastatic carcinoma nodule. Carcinoma cells show no thorium dioxide granules. × 400

be obtained before the proper therapy could be instituted were injected with thorotrast. Approximately 0.8 cc. of thorotrast per kilogram of body weight, divided into three doses, each given twenty-four hours apart, was injected in all cases. Twenty-four to forty-eight hours after the last injection, a plain antero-posterior radiograph was taken at a distance of 55 cm., using a potential of 90 K.V. and 50 milmps. for two seconds, with a Potter-Bucky diaphragm.

Clinically, little of note occurred at the time of injection which could be considered of a serious nature. Very slight rises of temperature or a sensation of tingling in various portions of the body were occasionally noted following the injections, but even these soon disappeared. Possibly, had more patients been injected, other symptoms noted by other observers may have appeared.

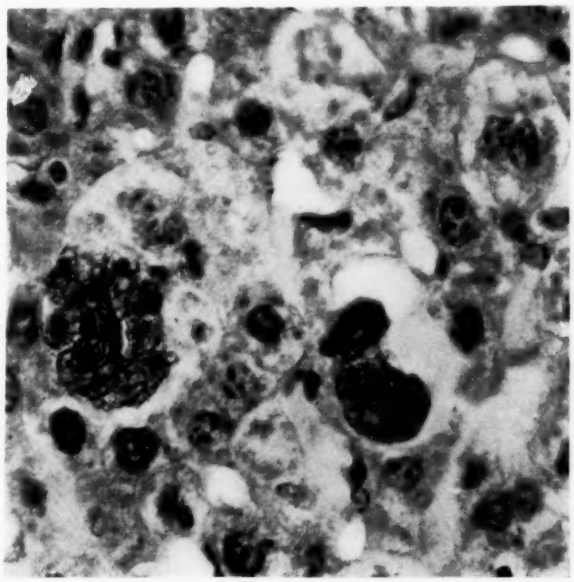

FIG. 8. LIVER OF DOG

Two years and two months following 0.8 cc. thorotrast per kilogram body weight. Aggregation of reticulo-endothelial cells filled with thorium dioxide particles nearly occluding the sinuses. × 450

The tissues of those patients who came to necropsy or operation subsequent to thorotrast injection revealed essentially the same histological pictures as described in the animal tissues, wherein the corresponding dosage was used. Four cases, in particular, presented interesting findings. One was that of a patient with carcinoma of the body of the pancreas and extensive metastases to the liver. The nodules of carcinoma cells, as diagnosed by the

areas of rarifaction in radiographs ante mortem, were free of any thorium dioxide particles. The surrounding liver substance showed the compressed reticulo-endothelial cells containing a considerable amount of thorium dioxide (fig. 7).

A patient with multiple myeloma with extensive bony metastases, yet no liver involvement, was studied. The myeloma nodules did not show the presence of thorium dioxide in any of

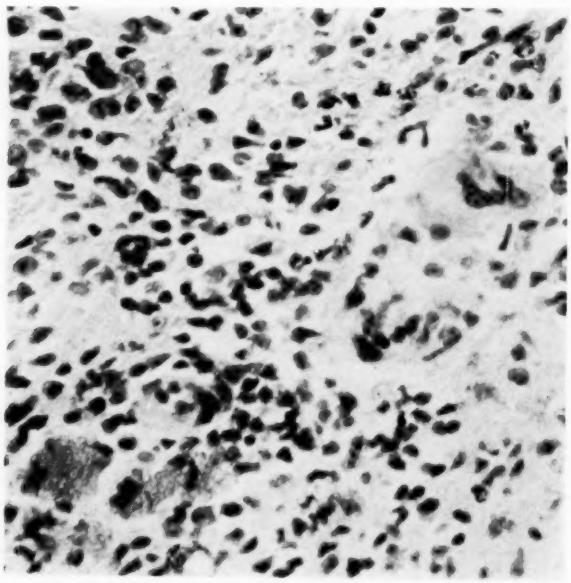

FIG. 9. SPLEEN OF DOG

Two years and two months following 0.8 cc. of thorotrast per kilogram body weight. Reticulo-endothelial cells filled with thorium dioxide particles. Presence of giant cells containing no thorium dioxide particles. × 500

the cells. Radiographs of the liver and spleen, ante mortem, revealed the liver to be homogenously smooth, no areas of rarifaction being noted.

A patient with multiple hydatid cysts of the liver, seen as well defined areas of rarifaction in the radiographs, was operated on and the cyst contents and wall mechanically removed. No thorium particles could be demonstrated in the clear cyst contents or the wall. This patient was markedly jaundiced at the time of

injection. However, after the cyst was drained and pressure removed, his jaundice disappeared. The draining sinus of the operative site has not completely healed as yet (fig. 11).

A fourth patient having an amebic abscess of the liver was operated on and the abscess drained, subsequent to thorotrast injection. The contents of the abscess and scrapings of the wall were examined. Vegetative forms of *Endamoeba histolytica* were demonstrated, but no thorium particles were found (fig. 10).

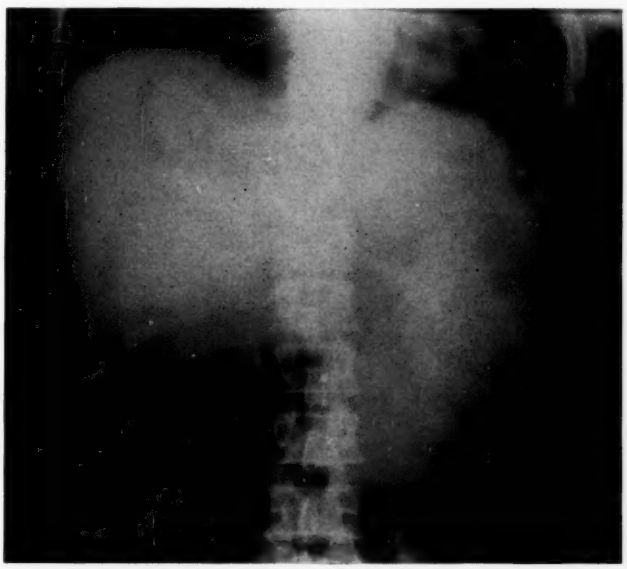

FIG. 10. RADIOGRAPH OF LIVER AND SPLEEN
Case of pernicious anaemia. Marked splenomegaly present

In the tissues studied at different times the thorium particles varied in size. The granules appeared larger in the cells of the tissues taken later after injection; possibly due to aggregation of the particles in the cells.

Identification of the thorium particles by specific chemical stains has not been possible so far. The granules appear as shiny, opaque, minute particles, simulating dark-colored, finely granular, bronze or the pigment of the malarial parasite in both unstained and stained preparations. At times, it is extremely

difficult to differentiate thorium dioxide particles from hemosiderin and other products of hemoglobin metabolisn, which are especially present in the spleen. A very useful method is the Berlin blue, or even better, Turnbull's blue tissue staining technique. The thorium particles remain unstained, but the hemosiderin is stained a beautiful clear blue. In my attempts to visualize lymph nodes by the subsutaneous injection of thorium

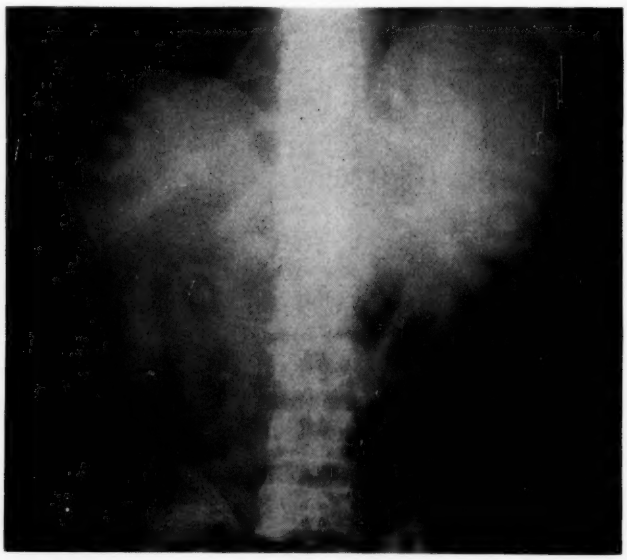

FIG. 11. RADIOGRAPH OF LIVER AND SPLEEN

Case of hydatid cyst of the liver oval area of rarefaction near lower right border clearly visible.

dioxide the draining normal nodes can be discerned rather clearly. However, those nodes in which the metastasizing malignant cells have completely occluded the draining sinuses, no thorium particles can enter, consequently the nodes do not increase their opacity to the roentgen rays. Microscopic examination of some of the nodes containing large malignant metastatic nodules, which were not visualized even after one year, revealed finely granular pigment simulating thorium dioxide. However, staining by the above methods revealed the fact that the particles were not thorium dioxide, but hemosiderin.

DISCUSSION

Upon analysis of this work and that of other investigators, it is strikingly notable, except in certain instances, that the most marked histopathologic changes are noted in the tissues of those animals which were given dosages larger than 0.8 cc. per kilogram of body weight. In other instances, the entire dose was given in one or only two injections, instead of in three or four fractions.

Our results as well as those of other investigators[2, 29] differ from those obtained by Shih and Jung. In the report of these latter investigators the manner of sacrificing the rabbits was not given nor was any note made concerning control rabbits killed by the same method. Possibly these notes may explain the difference in results. In our work, we did not note any regular instances of marked hemorrhagic tendencies or extravasations of blood into the internal organs. Dosages up to 8.0 cc. per kilogram of body weight divided into three doses, each given one day apart, were not accompanied by death in any instance, except when unsuccessful attempts at intracardiac (intraventricular) injection in the smaller animals resulted in extravasation of the solution into the heart muscle or mediastinum in one or two instances.

Our work, using the different doses, agrees in general with the recent results obtained by Whitaker, Davie and Murgatroyd. The instances of slight differences in results may be explained by the slight difference in dosages in the various animals.

Shute and Davis were not able to visualize the placenta of pregnant animals as reported by previous investigators. Certainly in our own sections, too few reticulo-endothelial cells are present in the placental tissues to fix sufficient thorium dioxide particles which will render the organ visible by the roentgen ray when doses of 0.8 cc. per kilogram of body weight are given. It appears from the work of these authors that doses up to ten times this amount are not sufficient to visualize the placenta roentgenographically. Coincidentally, as seen in the tissues of our experimental animals, intense degeneration of the liver and spleen parenchyma occurs when such large amounts of the substance is used. However, we did not note a single instance of rupture of

the spleen in any of our animals. Indeed, the mechanism of splenic rupture being caused by thorotrast injection would be difficult to explain in the light of present knowledge concerning the anatomy and physiology of the spleen. In the study of the spleen as a reservoir for red blood cells,[4] it was possible to watch the spleen under the fluoroscope in its dilated phase while the various animals were asleep under the influence of chloralose. One minute after the intravenous administration of adrenalin, contraction of the spleen could be seen very well; the degree of contraction being in accord with simultaneous hematocrit determinations.

The phase of the problem dealing with the radioactivity of thorium dioxide has been of considerable interest. This substance is the salt of the heavy metal thorium, which is classified among the radioactive metals. The Radium Institute of the Academy of Freiburg[11] found that 100 cc. of umbrathor which has the same thorium dioxide content as thorotrast contains a quantity of radioactive substance, the gamma-ray equivalent of which is that of the gamma-rays of 1.24×10^{-6} gr. of radium. Thus, since approximately only 50 to 60 cc. is used in the average patient, the entire liver and spleen, principally, are subjected to a total gamma-ray irradiation of 0.62×10^{-6} gr. of radium. As small as this may appear, Stewart and his associates reported that in one case, the spleen after autopsy, when placed on a photographic plate for one day, contained enough radioactive substance to register an image on the plate, while control spleens were negative. These results could not be confirmed by Yater and Otell[29] and Baumann and Schilling,[2] or my own work.

The results reported in this paper indicate that there is a distinct difference, both clinically and histopathologically, when different doses of thorotrast are used and that also the amount of clinical and histological reactions differ in instances wherein the substance is given in one large single dose or divided into three or four fractions, each given twenty-four hours apart.

Those animals whose tissues were examined two years after administration of thorotrast have revealed most interesting results. It is noted that the thorium dioxide granules are collected

almost entirely in the reticulo-endothelial cells which have aggregated in more or less focal areas, almost occluding the capillary sinuses. In the spleen, particularly, the presence of giant cells, not of the foreign body type, diffusely distributed throughout the organ is of distinct histopathologic interest. The results thus far indicate that the presence of the thorium dioxide particles are more or less permanent and the histopathologic changes although slight, are nevertheless present. The findings in the light of other histological pictures which are described can hardly lead one to believe that the method is "absolutely harmless" and can be used indiscriminately in all cases. These results are not in accord with the work of Radt.[19] This author reported that he had been unable to determine any histopathologic changes in the tissues of his animals even after one or two years following administration of thorium dioxide.

ADVANTAGES AND DISADVANTAGES OF THE METHOD

The advantages of the method may be summarized as follows:

(1) Distinct roentgenographic visualization of the liver and spleen is possible, using dosages of 0.8 cc. of thorotrast per kilogram of body weight intravenously. This has been a definite aid in differential diagnosis of abdominal masses or tumors, that is whether intrahepatic, intrasplenic or extrahepatic or extrasplenic.

(2) Metastatic or primary nodules in the liver or spleen when sufficiently large, may be discernible in the radiographs.

(3) Definite evidence of existing hydatid cysts, abscesses, cirrhosis and primary tumors in the liver and spleen can often be gained which evidence is hardly obtainable by any other method.

(4) Splenic motility, that is, contraction and dilatation as affected by various substances, can be studied in a more direct manner.

The disadvantages may be summarized as follows:

(1) A foreign substance is injected intravenously which is "fixed" by the reticulo-endothelial cells.

(2) Initial clinical reactions have been noted although these are neither consistent nor severe.

(3) Concomitant histopathologic changes, slight as they may appear, have definitely resulted in the reticulo-endothelial cells.

(4) The tissue studies, after two years, seem to indicate that the thorium particles remain more or less permanently in the reticulo-endothelial cells.

(5) Even should the radioactivity of the thorium dioxide particles be proved to be negligible, the mere presence of them as foreign bodies for so long a time, should not permit of the routine use of the method in cases other than the type noted.

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A NOTE ON ACETONE INSOLUBLE LIPOIDS IN RELATION TO ANTIGEN FOR THE WASSERMANN REACTION

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While the exact chemistry of alcoholic extracts of beef heart or other tissue in relation to antigen for the complement fixation and precipitation reactions in syphilis is as yet unknown, it is generally agreed that those lipoids soluble in alcohol and ether but insoluble in acetone possess a high degree of antigenic sensitiveness. These are believed to be mainly tissue lecithins or diaminomonophosphatids and are commonly referred to as "acetone insoluble lipoids." Curiously however, such lipoids extracted from egg-yolk or other substances do not possess the same sensitiveness as those derived from tissues so that the latter continue to be employed in the preparation of antigens for both the Wassermann and various precipitation procedures used in the serum diagnosis of syphilis.

In view of the remarkably high antigenic sensitiveness of these tissue acetone-insoluble lipoids it has occurred to us worth while to inquire into the possibilities of increasing the sensitiveness of antigen for the Wassermann reaction by endeavoring to prepare extracts carrying more than the usual amounts while keeping the cholesterol content at approximately 0.2 per cent in order not to increase the danger of falsely positive and non specific reactions attending the use of larger amounts of this sensitizing agent.*

When 25 grams of desiccated beef heart powder (Digestive Ferments Company) are extracted with 200 cc. of ether at room temperature for five days followed by extraction with 200 cc. of ethyl alcohol at 37°C., for four days, about 2.2 grams of acetone

* Unpublished data.

insoluble lipoids are recovered by precipitation of the ether and alcoholic extracts with an excess of acetone. When all of these along with 0.2 gram of cholesterol are dissolved in ether and added to a secondary alcoholic extract (100 cc.) of the tissue, the antigenic unit by the Kolmer method of titration is approximately 0.5 cc. of 1:2400. When 4.4 grams or double the amount of lipoids are added to 100 cc. of the secondary alcoholic extract along with 0.2 gram of cholesterol, the antigenic unit is raised to about 0.5 cc. of 1:2800 to 1:3000 with no increase of anticomplementary activity which is generally about 0.5 cc. of 1:4 for both antigens. However not all of the acetone insoluble lipoids in the second extract go into complete solution nor can solution be obtained by immersing the antigen in a water-bath at 56° to 75°C. as employed for aiding the solution of large amounts of cholesterol. But results have indicated that it is possible to increase the specific antigenic sensitiveness of extracts by increasing the amounts of acetone-insoluble lipoids.

When 2.2 grams of Pfanstiehl's ovo-lecithin was added to 100 cc. of a secondary alcoholic extract along with 0.2 per cent cholesterol, the antigenic unit was only 0.5 cc. of 1:800 while double this amount (4.4 grams) to 100 cc. of extract along with the same amount of cholesterol gave an antigenic unit of 0.5 cc. of 1:1200. It is apparent therefore that ovo-lecithin is not as antigenic as tissue lipoids; furthermore these extracts were sometimes slightly hemolytic in a dose of 0.5 cc. of 1:4 but showed no increase of anticomplementary activity.

In Noguchi's later method of preparing acetone-insoluble lipoids, beef heart is first extracted with acetone to remove hemolytic and anticomplementary substances and the dried residue then extracted with absolute ethyl alcohol to secure the alcohol soluble lipoids. In one experiment we extracted 30 grams of desiccated beef heart (Digestive Ferments Company) with 100 cc. of acetone for five days at room temperature. The acetone was discarded, the tissue dried and extracted with 100 cc. of absolute ethyl alcohol for five days at room temperature. A portion of this extract was sensitized with 0.2 per cent cholesterol

and was found to give an antigenic unit of 0.5 cc. of 1:6000 by the Kolmer method of titration. A second portion of 50 cc. was evaporated by fanning to 25 cc. to concentrate the lipoids and sensitized with 0.2 per cent cholesterol. This extract had a slight sediment of undissolved lipoids but gave an antigenic unit of 0.5 cc. of 1:8000 which appeared to be due to the higher concentration of alcohol soluble lipoids since the amount of cholesterol was the same in both. Neither extract was hemolytic in dose of 0.5 cc. of 1:4 and the anticomplementary unit of each was 0.5 cc. of 1:4 so that an increase of antigenic sensitiveness was secured without an increase of non specific complement fixation.

Somewhat similar results were obtained by extracting 25 grams of the desiccated beef heart with 100 cc. of absolute acetone free ethyl alcohol for five days at room temperature without the preceding extraction with acetone. When a portion of this extract was sensitized with 0.2 per cent cholesterol, the antigenic unit was found to be 0.5 cc. of 1:6000. When 40 cc. was concentrated to 20 cc. by fanning and sensitized with 0.2 per cent cholesterol there remained a slight residue of undissolved lipoids but the antigenic unit was increased to 0.5 cc. of 1:8000. Both extracts were entirely free of hemolytic and anticomplementary activity in amounts as high as 0.5 cc. of 1:4.

It would appear possible therefore to increase the sensitiveness of antigens for the Wassermann test by increasing the amounts of lipoids contained in alcoholic tissue extracts without increasing the amount of cholesterol beyond 0.2 per cent which is inadvisable at least in those methods employing a primary incubation of fifteen hours or longer at 6°C.* Furthermore we have observed that such antigens, as briefly described herein, may be used in a dose of 20 antigenic units in the Kolmer modification of the Wassermann test with increased antigenic sensitiveness and with no falsely positive or non specific reactions with non syphilitic sera since such a dose is still from forty to eighty times less than the anticomplementary amounts and representing therefore an extremely wide and safe range.

* Unpublished data.

CONCLUSIONS

(1) It is possible to increase the specific antigenic sensitiveness of alcoholic extracts of beef heart by increasing the amounts of alcohol soluble but acetone insoluble lipoids.

(2) Such extracts sensitized with no more than 0.2 per cent cholesterol possess a very high degree of antigenic sensitiveness with no increase of non specific or anticomplementary properties.

(3) Antigens of this kind permit the use of larger amounts in conducting the Wassermann test with an increase of specific sensitiveness for syphilis antibody.

EDITORIAL

"Yaws and Syphilis. Two Diseases or One?"

Under the above caption, D. B. Blacklock, M.D., Professor of Parasitology, School of Tropical Medicine, University of Liverpool, gives such a well-reasoned and convincing analysis of this vexed question that the Editor of Tropical Diseases Bulletin, in the November, 1933 issue in which his paper is published, is moved to remark in a foot-note as follows: "Professor Blacklock questions the soundness of much of the current beliefs about yaws and its relation to syphilis. His arguments will doubtless receive open-minded attention."

In a paper published by the undersigned in collaboration with Lieut. Comdr. Edwin Peterson (MC) U. S. Navy some five years ago, one of the conclusions arrived at was that medicine was already in possession of sufficient facts to settle this matter in favor of unity (Professor Blacklock uses the term "unicity," a word in use when gonorrhoea was also thought to be a part of syphilis), if only we would apply a little logical reasoning to these known facts. Professor Blacklock has applied this logic with meticulous precision.

In his estimable work, "Diagnostics and Treatment of Tropical Diseases," Fifth Edition published by P. Blakiston's Son & Co. in 1929, Admiral Stitt used the same technique as Professor Blacklock, that is, he took the table of points purporting to differentiate syphilis and yaws and defeated "duality" in detail. In addition, Stitt published pictures from known yaws cases, showing aneurysms of the aorta and detailing findings in framboesia which completely stultified the differential tables. Tropical Diseases Bulletin in its review of Stitt's book at the time of the appearance of this Fifth Edition, after observing that it would find its way on to the shelves of all American (sic!)

practitioners in the tropics, used the following words in describing his treatment of yaws:

> The author, influenced by workers in Haiti, has elected to accept the evidence in favour of the identity of yaws and syphilis, which in the present state of our knowledge seems unwise. He then describes yaws shorn of some of its well known characteristics but clothed in rather unusual garb decked out with visceral lesions, hepatic gummata, aneurysms and cerebral haemorrhage. The text bears evidence of rather hasty compilation and critical analysis reveals numbers of minor omissions and little inaccuracies and some looseness of expression. After such a statement it is only fair to give examples of these blemishes which will no doubt disappear in subsequent editions. Secondary yaws lesions on the trunk are said to be rare, which is not true.

After this rather rollicking review, it will be a source of satisfaction to physicians everywhere that Stitt's views are beginning to find justification even in quarters formerly hostile to them.

It is to be regretted that the Profession in Great Britain had to permit a logician to show them the folly of their course in fighting over this question. It has already been pointed out* that

> Yaws then means a certain definition, fallacious withal, into which must be crowded all those cases of syphilis which appear to omit certain well-recognized symptoms of the European disease.

From the days in the seventeenth century when Thomas Sydenham sized up this matter correctly down to the present time, every generation has seen one or more English physician whose research and reasoning were correct upon the venereal diseases. The names of Benjamin Bell, Berkeley Hill, and Jonathan Hutchinson, to mention only three, will forever be remembered when the venereal diseases are under discussion. The last named was not only one of the greatest syphilographers of all time but his brand of logic when trained upon the yaws-syphilis question left nothing to be desired. It should not have taken all this time for truth to prevail. The acceptance of truth is, however, a slow process oftentimes. The rancor engendered by the hero-worshippers requires time to die down. Professor Blacklock quotes many

* Butler, C. S.: Diagnosis and treatment of Yaws. Internat. Clinics, ser. 40, **2**: 1–14. 1930.

more or less important writers along the line of the yaws-syphilis investigations but has little to say about his own countrymen who have born the brunt of it in defence of what they knew to be true. Nor aught but silence has he for the group of Americans, principally U. S. Naval Medical Officers, who have, by research and writing, defended Hutchinson's views for the past thirty years.

The challenge issued in the Lancet of April 25, 1931 is still in order. Here it is: "Yaws is purely an artefact over the disease syphilis. This statement is made with the hope that some dualist will produce evidence to the contrary." There has been ample time for all such evidence to assert itself.

—C. S. Butler.

NEWS AND NOTICES

Further Experience with the Friedman Hormone Test for Pregnancy

The Committee on Research is pleased to make the following report concerning further experience by members of the American Society of Clinical Pathology with the Friedman hormone pregnancy test.

From the reports, there is evidently a tendency for the rabbits to be observed after a forty-eight hour period instead of a twenty-four hour period as first proposed for the test. Some men inject the rabbits more than once and many rabbits are observed at operation and used again later. Table 1 is a compilation of results reported during 1933 and there is combined with these reports those previously reported. This makes a grand total of 5,759 cases in which there were 152 erroneous results or 2.63 per cent. There is a 1 per cent error in reporting positives and 4.6 per cent error in reporting negatives; 0.3 per cent were reported as doubtful. This indicates that the test is accurate in 97 per cent of the trials. The test shows up well in chorioepitheliomas and hydatid moles where twenty-five out of twenty-six cases were positive. The instance of positive tests in ectopic pregnancy is 77.6 per cent for the combined groups. About 1.5 per cent of the animals die before the test is completed.

Table 2 indicates the tests which have been run in an attempt to diagnose tumors of the testis. Ferguson, in a recent paper, has pointed out the inadvisability of using the rabbit in testing for this condition and demonstrated the necessity of a quantitative test. Nevertheless, it is interesting to note that in eight instances out of nineteen trials, the test was positive.

Many members of the American Society of Clinical Pathology have received a complimentary copy of "Dehydrated Culture Media and Reagents" issued by the Difco Laboratory of Detroit,

TABLE 1

FRIEDMAN HORMONE PREGNANCY TESTS

TOTAL TESTS	POSITIVE	NEGATIVE	FALSE POSITIVE	FALSE NEGATIVE	ECTOPIC-POSITIVE	ECTOPIC-NEGATIVE	CHORIOEPITHELIOMA OR HYDATID MOLE-POSITIVE	CHORIOEPITHELIOMA OR HYDATID MOLE-NEGATIVE	EARLIEST TEST. DAYS AFTER LAST MENSTRUATION	AMOUNT OF URINE (EACH INJECTION)	NUMBER OF INJECTIONS	DURATION OF TEST	ANIMALS DYING FROM INJECTIONS	DOUBTFUL	CLINICAL PATHOLOGIST REPORTING
13	6	6							11	*cc.*		*hours*		1	Dr. R. C. Beck, Richmond, Virginia
169	89	77		1	1		1	*	33	8–10	3–4	36–48	3	3	Dr. A. G. Foord, Pasadena Hospital, Pasadena, California
9	4	5							10	10	2	96			Dr. H. A. Heise, Uniontown, Pennsylvania
86	77	9							10						Dr. G. B. Kramer, Youngstown Hospital, Youngstown, Ohio
109	61	48			4		1		33	12[1]	1	36–48	1		Dr. Seab J. Lewis, Beaumont, Texas
67	31	36	1	5	2				29	10	3[2]	48	3		Dr. O. W. Lohr, Saginaw, Michigan
109	42	67		2					7						Dr. J. M. Moore, San Antonio, Texas
16	11	5							21						Dr. H. R. Prentice, Bronson Hospital, Kalamazoo, Michigan
289	129	160	5	4	4	4	4		8	10	2[3]	48	8		Dr. W. M. Simpson, Miami Valley Hospital, Dayton, Ohio
37	16	21							10	10	1	48			Dr. M. Warren, Maine General Hospital, Portland, Maine
260	134	121	2[4]	2	2		1		31	10	2	48	7	5	Dr. A. M. Young, Mount Sinai Hospital, Cleveland, Ohio
1,164	600	555	8	14	13	4	7						22	9	
4,595	2,526	2,058	24	106	88	25	18	1					72	11	Previously reported—Am. Jour. Clin. Path., **3**: 97–102, 1933
5,759	3,126	2,613	32	120	101	29	25	1					94	20	Grand total

* Two tests negative in patients with hydatid mole after operation. Two tests negative in patients with chorioepithelioma after operation.

[1] Catheterized specimens.

[2] Twelve hours apart.

[3] Six hours apart.

[4] One case teratoma ovary, 1 case tuberculosis of genital tract.

Michigan. This is the fourth edition of this extremely useful manual and copies of it may be procured by addressing the Director of the Laboratory.

Dr. J. H. Black is Chairman of a committee engaged in revising the Constitution and By-laws of the Society. Any member of the

TABLE 2

FRIEDMAN TESTS IN TUMORS OF THE TESTIS

TESTS DONE	POSITIVE	NEGATIVE	REMARKS	CLINICAL PATHOLOGISTS REPORTING
2	1	1	Positive in case of tumor removed 12 days previously, negative in case of seminoma removed 2 years previously.	Heise
2	2		1—teratoma testis; 1—seminoma testis.	Lohr
7	3	4	3 positive in cases of embryonal carcinoma of testis—(microscopic diagnosis), 4 negatives in suspected malignancy testes.	Simpson
2	2		2 tests on same patient with teratoma testis.	Young
4		4	3 cases of seminoma, 1 case of teratoma testis.	Foord
1		1	2 months after operation for teratoma testis.	Lewis
1		1	2 days after operation for teratoma testis.	Moore
19	8	11		

Society who has any suggestions to make concerning the Constitution and By-laws is urged to get in immediate communication with Dr. Black.

The Thirteenth Annual Convention of the American Society of Clinical Pathologists will be held in Cleveland on June 8, 9, and 10. The Headquarters is the Hotel Cleveland. The manager of the Hotel will send members of the Society returnable postcards for reservations. It is urged that these reservations be made as promptly as possible. The Hotel has set aside 150 rooms for members of the Society which will be held until April 1.

The Program Committee has made up a very fine program for the Convention, a feature of which will be "Cancer Diagnosis." It is hoped that a large attendance will be present.

Members are urged to make early application for space for their scientific exhibits in order that the local committee may make proper arrangements. Members desiring to read papers on the program should forward their titles to the Secretary not later than April first.

Drs. Warren T. Vaughan and Walter Simpson have been appointed to the Editorial Board of the Journal. They replace Drs. Keilty and Lynch whose terms expire.